Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

OCT 1 8 2011

510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is **K111904**.

807.92 (a)(1): Name: **ARK Diagnostics, Inc.**

Address: 1190 Bordeaux Drive
Sunnyvale, CA 94089

Owner Operator Number: 10027663
Establishment Registration: 3005755244

Phone: (408) 747-0700
FAX: (408) 747-0783
Contact: Kenneth C. Kasper, PhD – (408) 747-0708
Executive Director of Quality and Regulatory Affairs

Date prepared: September 7, 2011

807.92 (a)(2): Device name- trade name and common name, and classification

Trade name: ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control

Common Name: Homogeneous Enzyme Immunoassay

Classification: 21 CFR 862 Clinical Chemistry Test System - Toxicology; Test Code LAO; Enzyme Immunoassay, Methotrexate Pre-Amendment Device, Unclassified
(21 CFR 862.3200 DLJ, 21 CFR 862.3280 LAS)

807.92 (a)(3): Identification of the legally marketed predicate device

Abbott TDx®/TDxFLx® METHOTREXATE II (K932615)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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807.92 (a)(4): Device Description

The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and Methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

The ARK Methotrexate Assay consists of reagents R1 anti-Methotrexate polyclonal antibody with substrate and R2 Methotrexate labeled with bacterial G6PDH enzyme. The ARK Methotrexate Calibrator consists of a six-level set to calibrate the assay, and the ARK Methotrexate Control consists of a six-level set used for quality control of the assay (tri-level calibration range set and tri-level high range set). The ARK Methotrexate Dilution Buffer is equivalent to zero calibrator (Calibrator A).

807.92 (a)(5): Intended Use / Indications for Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK Methotrexate Calibrator is intended for the calibration of the ARK Methotrexate Assay.

The ARK Methotrexate Control is intended for the quality control of the ARK Methotrexate Assay.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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807.92 (a)(6): Technological Similarities and Differences to the Predicate

SUBSTANTIAL EQUIVALENCE COMPARATIVE CHART

Comparison between the ARK™ Methotrexate Assay and TDx®/TDxFLx® Methotrexate II Assay

Characteristic	Device ARK™ Methotrexate Assay	Predicate Abbott TDx®/TDxFLx® Methotrexate II (K932615)
Intended Use	The ARK™ Methotrexate Assay is intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers.	The TDx®/TDxFLx® Methotrexate II assay is a reagent system for the quantitative measurement of methotrexate, an antineoplastic drug, in serum or plasma.
Indications for Use	The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.	The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.
Sample	Serum or plasma	Serum or plasma
Methodology	Homogenous enzyme immunoassay (EIA)	Fluorescence polarization immunoassay (FPIA)
Reagent Components	Two (2) reagent system: Anti-Methotrexate Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to Methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers Enzyme Reagent (R2) containing Methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	Reagent Pack: W = Wash solution, solvent, sodium azide S = Methotrexate Antibody (mouse monoclonal), buffer, protein and sodium azide T = Methotrexate Fluorescein Tracer, buffer, protein, surfactant and sodium azide P = Pretreatment Solution, protein, surfactant and sodium azide
Platform required	Automated clinical chemistry analyzer	TDx clinical chemistry analyzer
Accessory reagents	Calibrators (six levels) and controls (six levels) in a synthetic matrix; Dilution Buffer	Calibrators (six levels) and controls (six levels) in human serum; Dilution Buffer
Testing environment	Routine clinical laboratory	Routine clinical laboratory
Reagent condition and storage	Liquid, 2-8° C	Liquid, 2-8° C

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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807.92 (b)(1) and 807.92 (b)(2):
Brief Description of Nonclinical and Clinical Data

Limit of Quantitation (LOQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

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Accuracy

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Target concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0:10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Assay Range

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the measurement range.

Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (monoclonal FPIA).

Methotrexate concentrations by FPIA ranged 0.04 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.04 to 1500 µmol/L. Results of the Passing-Bablok regression analysis for the study are shown below (with 95% confidence limits) for 102 specimens within the measurement range as well as for all 147 specimens including those above the measurement range requiring dilution.

Parameter	**Range 0.04 to 1.19 µM**		**Range 0.04 to 1440 µM**	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.00 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.978	(0.968 to 0.985)	0.998	(0.997 to 0.998)
Number of Samples	102	na	147	na

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within-run, between-day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.10 µmol/L, SD ≤0.01 at ≤0.10 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		Methotrexate (~ 0.50 µmol/L)	
Interfering Substance	**Interferent Concentration**	**Serum Control**	**Test**	**Serum Control**	**Test (% Control)**
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity - Drug Interference

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-Hydroxymethotrexate.

Crossreactivity to the minor, inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA)

The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for methotrexate cancer treatement due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Compound	Tested (µmol/L)	Methotrexate Absent MTX (µmol/L)	Methotrexate Absent Cross Reactivity (%)	Methotrexate Present 0.05 µmol/L MTX (µmol/L)	Methotrexate Present 0.05 µmol/L Cross Reactivity (%)	Methotrexate Present 0.50 µmol/L MTX (µmol/L)	Methotrexate Present 0.50 µmol/L Cross Reactivity (%)
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

Anticoagulants

Studies were conducted to determine the performance characteristics of the assay for both serum and plasma samples containing methotrexate.

The results indicate that there is no significant difference between the recovery of methotrexate in serum or plasma.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Sample Stability

Human specimens were shown to be stable frozen (at least 15 months), forty-eight (48) hours at room temperature, refrigerated (at least 21 days) and after three (3) successive freeze/thaw cycles.

On-Board Stability

Calibration Curve Stability: A stored calibration was effective up to at least 19 days based on supporting data.

Reagent on-board stability: Reagents were effective when stored after transfer to analyzer specific reagent containers for up to at least 25 days based on supporting data. In-use stability of calibrator and controls was also demonstrated.

807.92 (b)(3): Conclusions from Nonclinical Testing

As summarized above, the ARK Methotrexate Assay System, including the ARK Methotrexate Calibrator, ARK Methotrexate Control and ARK Methotrexate Dilution Buffer, is substantially equivalent to the Abbott TDx®/TDxFLx® METHOTREXATE II Assay system. The ARK Methotrexate Assay system was shown to be safe and effective for its intended use based on performance studies.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

OCT 18 2011

ARK Diagnostics, Inc
c/o Kenneth C. Kasper, PhD
1190 Bordeaux Dr.
Sunnyvale, CA 94089

Re: k111904
Trade/Device Name: ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, ARK™ Methotrexate Control
Regulatory Class: Unclassified, 510(k) required
Product Code: LAO, DLJ, LAS
Dated: September 7, 2011
Received: September 8, 2011

Dear Dr. Kasper:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at (301) 796-5760. For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-5680 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Indication for Use

510(K) Number (if known): K111904

Device Name:

ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control

Indications for Use:

The ARK™ Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of Methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK™ Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay.

The ARK™ Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Prescription Use ___X___ (21 CFR Part 801 Subpart D)	And/Or	Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Ruth Chesler

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) 111904

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Services
Food and Drug Administration

Memorandum

Date: 10-10

From: DMC (HFZ-401)

Subject: Premarket Notification Number(s): K111904/A2

To: Division Director: TX/OCTD

The attached information has been received by the 510(k) DMC on the above referenced 510(k) submission(s). Since a final decision has been rendered, this record is officially closed.

Please review the attached document and return it to the DMC, with one of the statements checked below.

_____ Information does not change the status of the 510(k); no other action required by the DMC; please add to image file. (Prepare K-25) THIS DOES NOT APPLY TO TRANSFER OF OWNERSHIP. PLEASE BRING ANY TRANSFER OF OWNERSHIP TO POS.

_____ Additional information requires a new 510(k); however, the information submitted is incomplete; (Notify company to submit a new 510(k); [Prepare the K30 Letter on the LAN]

__✓__ No response necessary (e.g., hard copy of fax for the truthful and accuracy statement, 510(k) statement, change of address, phone number, or fax number).

CLIA CATEGORIZATION refers to laboratory test system devices reviewed by the Division of Clinical Laboratory Devices (HFZ-440

_____ Information requires a **CLIA CATEGORIZATION**; the complexity may remain the same as the original 510(k) or may change as a result of the additional information (Prepare a CAT letter)

_____ Additional information requires a **CLIA CATEGORIZATION**; however, the information submitted is incomplete; (call or fax firm)

__✓__ No response necessary

This information should be returned to the DMC within 10 working days from the date of this Memorandum.

Reviewed by: Matthew Lyn

Date: January 10, 2013

✓

DLC 1/17

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

K111904/92

ARK Diagnostics, Inc.
Quality health through drug management

October 02, 2012

FDA CDRH DMC
OCT - 4 2012
Received

Document Mail Center (WO66-0609)
OIVD/CDRH/FDA
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

RE: Revised Labeling – ARK Diagnostics, Inc.
Establishement Registration No. 3005755244
Owner Operator No. 10027663

Dear FDA Staff,

ARK Diagnostics submits copies of its finished labeling that were revised due to relocation of the manufacturing site. The establishment registration profile has been updated. The appended documents contain copies of container labels and package inserts for the following IVD medical devices. Labeling changes apply to the version date, August 2012, address and fax number, and respective revision numbers. US Patent information was added in Section 14 for the Levetiracetam Assay. Also, Global Trade Identification Numbers (GTIN) were added to the kit labels.

Medical Device	**510(k) File Number**
ARK Gabapentin Assay	K101574
ARK Lamotrigine Assay	K101305
ARK Levetiracetam Assay	K091653
ARK Methotrexate Assay	K111904 ✓
ARK Topiramate Assay	K083799
ARK Zonisamide Assay	K091884

Please feel free to contact me if you have any questions, or require additional information, and please note my contact information below.

Sincerely,

KC Kasper

Kenneth C. Kasper, Ph.D., RAC
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.
Tel 510-270-6280
e-mail: ken@ark-tdm.com

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

RE: K111904

ARK Diagnostics, Inc.

Doc. No. 0743-0026-00 Rev. No. 04

CN No. 12-025

PROCESS GUIDELINE Effective Date: 9/11/12

Label Proof – Methotrexate Products

1. PURPOSE

This document comprises the label proofs for comparison of raw material stock labels for the Methotrexate assay products, reagents, calibrators, controls and dilution buffer. The label proofs are actual, approved pre-printed stock labels. It also comprises appended copies of the current approved version of package inserts to facilitate material inspection.

2. SCOPE

The process guideline applies to the inspection of raw material stock labels and package inserts for Methotrexate assay products at ARK Diagnostics, Inc (ARK), Fremont.

3. REFERENCES

0740-0026-01 – Purchase Requisition for Methotrexate Package Inserts
0743-0001-00 – Receiving and Inspection
0743-0001-01 – Receiving Purchase Order - Inventoried Item
0900-0003-38 – Product Label Printing In-House
0900-0004-23 – Specification, ARK Methotrexate Package Inserts
0900-0004-24 – Label Proofs – Methotrexate Assay

4. RESPONSIBILITIES

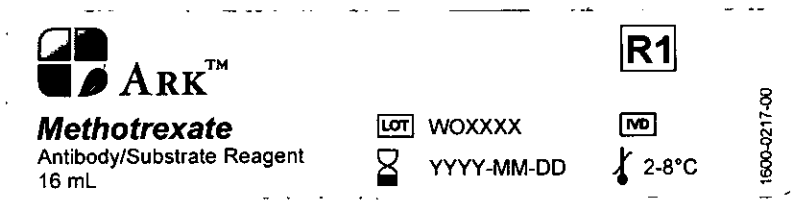
OPERATIONS and QA are responsible for maintaining this guideline.

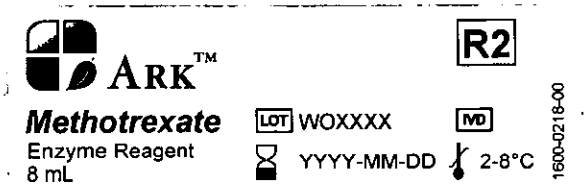
5. PROCEDURE

5.1. Container Labels for Methotrexate assay products are produced according to Doc. No. 0900-0003-38 – Product Label Printing In-House. Compare the label for its respective product.

5.2. Consult this label proof for each label part number. Verify the lot number and expiration date of produced labels according to the Production Order and printing procedure. This label proof is comprised of labels with generic lot number **WOXXXX** and expiration date **YYYY/MM/DD**.

5.3. Bottle Labels for Methotrexate Reagents



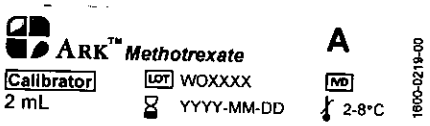


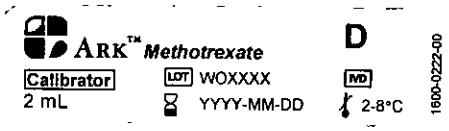
Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

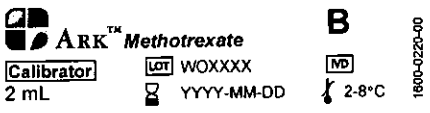
Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

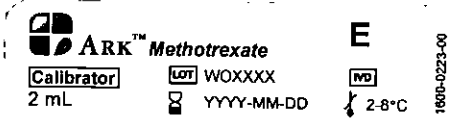
Label Proof – Methotrexate Products

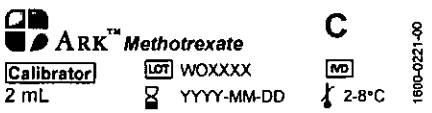
5.4. Bottle Labels for Methotrexate Calibrators

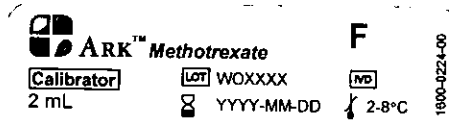




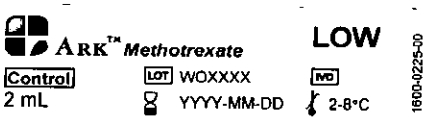


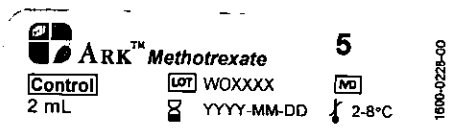


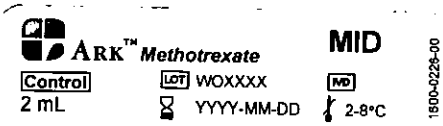


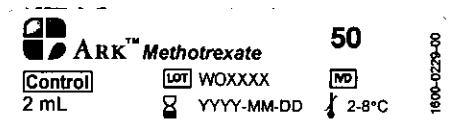


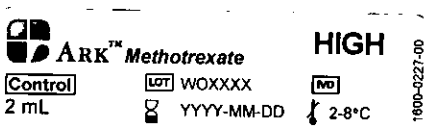
5.5. Bottle Labels for Methotrexate Controls

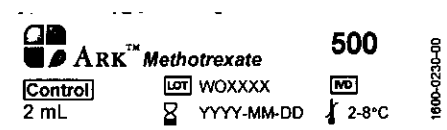












Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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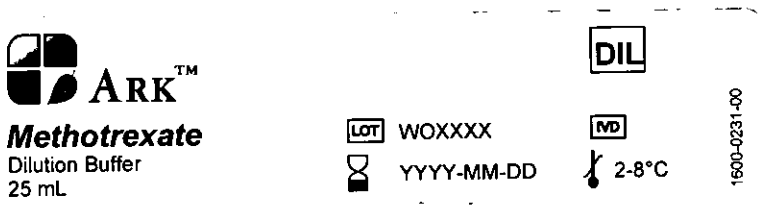
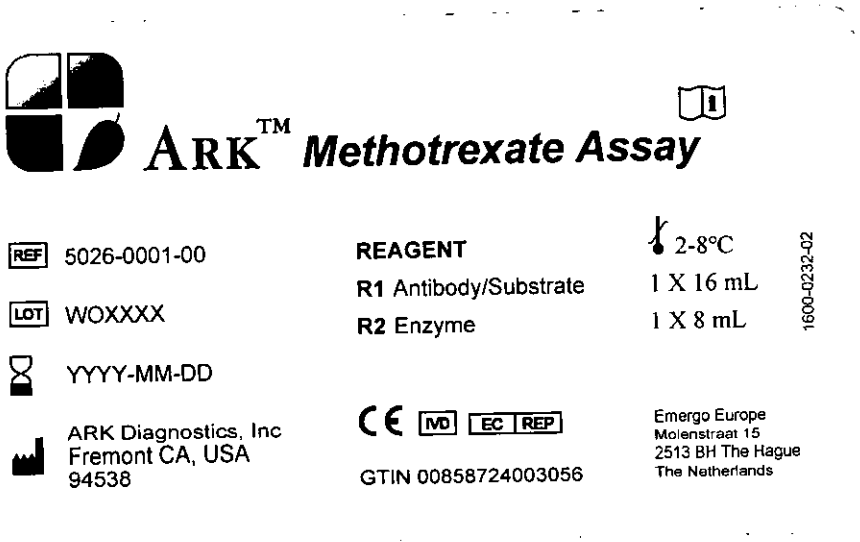
Label Proof – Methotrexate Products

5.6. Bottle Label for Methotrexate Dilution Buffer



5.7. Kit Label for Methotrexate Reagents



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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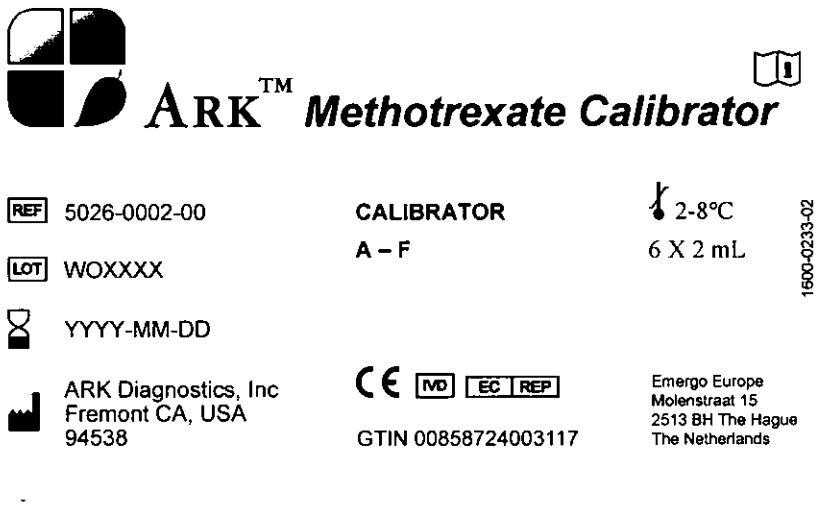
Label Proof – Methotrexate Products

5.8. Kit Label for Methotrexate Calibrators



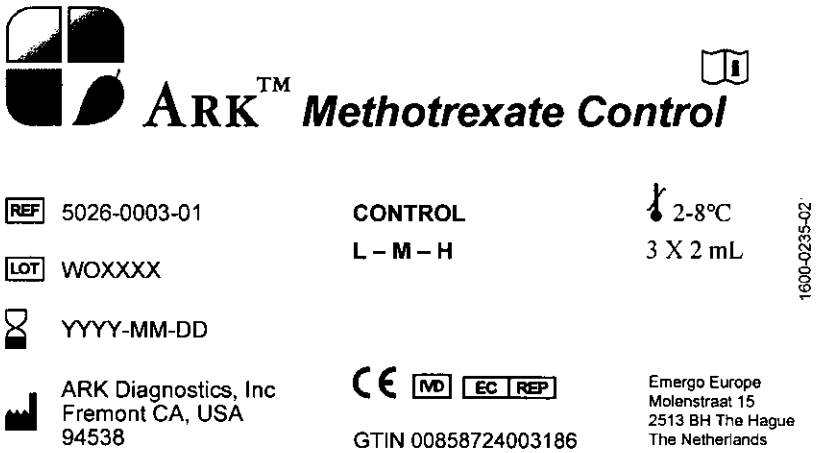
5.9. Kit Label for Methotrexate Controls [complete set]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Label Proof – Methotrexate Products

5.10. Kit Label for Methotrexate Controls [Calibration Range set]



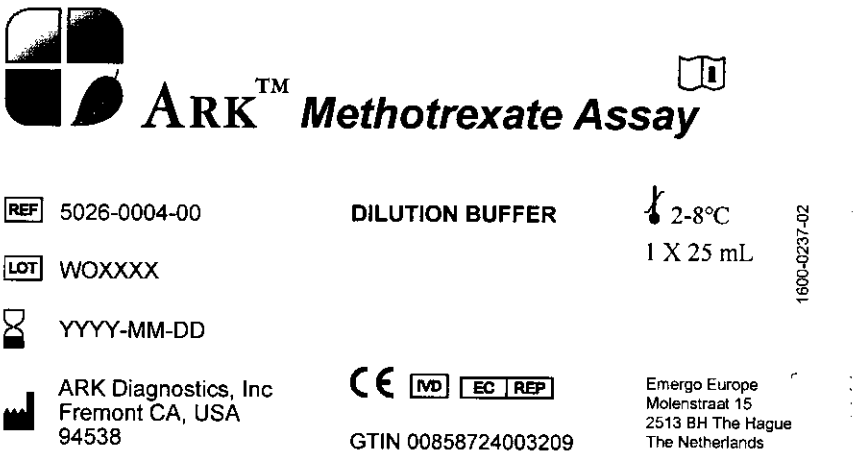
5.11. Kit Label for Methotrexate Controls [High Range set]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Label Proof – Methotrexate Products

5.12. Kit Label for Methotrexate Dilution Buffer



5.13. Appended copies of package inserts:

- 1600-0213-00 – August 2012, Rev04, Methotrexate Assay
- 1600-0214-00 – August 2012, Rev03, Methotrexate Calibrator
- 1600-0215-00 – August 2012, Rev03, Methotrexate Control
- 1600-0216-00 – August 2012, Rev03, Methotrexate Dilution Buffer

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Aclamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-5-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
Fluorouracil	3000
Mercaptopurine	1000
10-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1800
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

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14 TRADEMARKS

ARK™ is a trademark of ARK Diagnostics, Inc.

Other brand or product names are trademarks of their respective holders.

ARK Diagnostics, Inc.
Fremont, CA 94538 USA

Printed in USA
Revised August 2012
1600-0213-00 Rev 04

ARK Diagnostics, Inc.

ARK™ *Methotrexate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.
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Fremont, CA 94538 USA
Tel: 1-877-869-2320
Fax: 1-510-270-6298
customersupport@ark-tdm.com
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CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel. (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2

© 2012, ARK Diagnostics, Inc.

Reagent Kit REF 5026-0001-00

1 NAME

ARK™ *Methotrexate Assay*

2 INTENDED USE

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

3 SUMMARY AND EXPLANATION OF THE TEST

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino]benzoyl]-L-glutamic acid], formerly Amethopterin, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1,2] Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Guidelines for methotrexate therapy with leucovorin rescue should be consulted.[1] Intermediate to high doses of methotrexate (approximately 35 mg/m² - 12 g/m²) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[3,4]

4 PRINCIPLES OF THE PROCEDURE

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENT

REF	Product Description	Quantity/Volume
5026-0001-00	**ARK Methotrexate Assay**	
	Reagent R1 – Antibody/Substrate rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative, and stabilizers	1 X 16 mL
	Reagent R2 – Enzyme methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative, and stabilizers	1 X 8 mL

Reagent Handling and Storage

ARK Methotrexate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

6 WARNINGS AND PRECAUTIONS

- For **In Vitro Diagnostic** Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice.
- The sampling time of methotrexate will be dependent on dose, duration of infusion and clinical status of the patient. Consult specific treatment protocols for sampling time.
- Whole blood cannot be used. The following anticoagulants may be used with this assay:
  - Sodium heparin
  - Lithium heparin
  - Potassium EDTA
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- Clarified specimens may be stored up to two weeks at 2 to 8°C. If testing will be delayed, specimens may be stored frozen (≤ -10°C) prior to being tested. Care should be taken to limit the number of freeze-thaw cycles. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C.
- **Handle all patient specimens as if they were potentially infectious.**

Records released by CRH on 02-22-2016 under Request # 2015-888

8 PROCEDURE

Materials Provided
ARK Methotrexate Assay – REF 5026-0001-00

Materials Required – Provided Separately
ARK Methotrexate Calibrator – REF 5026-0002-00
Quality Controls – ARK Methotrexate Control – REF 5026-0003-00
ARK Methotrexate Dilution Buffer REF 5026-0004-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Calibration
Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E and F, test calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Quality Control (QC)
Laboratories should establish QC procedures for the ARK Methotrexate Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol
The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L) are assayed by dilution of the specimen and controls into the measurement range.

Manually dilute the high specimen or control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

Sample Volume	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

Manual Dilution Factor = (Volume of Specimen + Volume of Dilution Buffer) / Specimen Volume

Multiply the assayed result by the dilution factor.

9 RESULTS
To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

10 LIMITATIONS OF PROCEDURE
This assay is designed for use with serum or plasma only; refer to the sections Specimen Collection and Preparation for Analysis. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variability. See the section Expected Values below.

As with all analyte determinations, the methotrexate value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

IMPORTANT: Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should not be tested with the ARK Methotrexate Assay. These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid (DAMPA)[10-12] that result from metabolism of methotrexate by glucarpidase. DAMPA crossreacts with the methotrexate antibody used in this assay, and may continue to circulate for at least five to seven days before accurate measurements of serum methotrexate may return.[13] Oncologists on the clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA that would confuse the efforts of glucarpidase therapy.[13] While glucarpidase is well tolerated and rapidly reduces circulating MTX, delayed renal elimination of MTX can still be a problem for adult and elderly patients.[14]

11 EXPECTED VALUES
Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors.[1,2] While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example),[15] concentrations may exceed 1000 µmol/L[16] with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.[17] For treatment of osteosarcoma,[18] the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, less than 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin in accordance with algorithms until the MTX level is <0.1 µmol/L. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.[1,9] Some centers follow ≤ 0.10 µmol/L.[16,19]

From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate,[1,9,16]

Clinical Situation	Laboratory Findings	
	Methotrexate Level (µmol/L)	Hours after administration
Normal Methotrexate Elimination	~10	24
	~1	48
	<0.2	72
Delayed Late Methotrexate Elimination	>0.2	72
	>0.05	96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50	24
	≥5	48
	OR	
	≥100% increase in serum creatinine	24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs,[14,16] for example vancomycin.[20] Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.[21-26]

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite,[15,27] its molar ratio to methotrexate of up to approximately 100-fold,[28] and relative insolubility versus the parent drug,[14,19] possible nephrotoxicity due to precipitation of the metabolite in renal tubules[29] may delay elimination of methotrexate itself.

Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.[14] Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.[13]

12 SPECIFIC PERFORMANCE CHARACTERISTICS
Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche/Hitachi 917 System.

Limit of Quantitation (LoQ)
The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

Measurement Range
The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the measurement range.

Recovery
Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

% Recovery = 100 X Mean recovered concentration / Theoretical concentration

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.00	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

Linearity
Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.006 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

Method Comparison
Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (monoclonal FPIA)[30].

Methotrexate concentrations by FPIA ranged 0.04 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.04 to 1500 µmol/L. Results of the Passing-Bablok[31] regression analysis for the study are shown below (with 95% confidence limits) for 102 specimens within the measurement range as well as for all 147 specimens including those above the measurement range requiring dilution.

Parameter	Range 0.04 to 1.19 µM		Range 0.04 to 1440 µM	
Slope	1.00	(1.00 to 1.02)	0.99	(0.98 to 1.00)
y-intercept	0.01	(0.00 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.978	(0.968 to 0.985)	0.998	(0.997 to 0.998)
Number of Samples	102	na	147	na

Precision
Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.1 µmol/L, SD ≤0.01 at ≤0.1 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.4
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.2
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

Interfering Substances
Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

Interfering Substance	Interferent Concentration	Methotrexate (~ 0.05 µmol/L) Serum Control	Methotrexate (~ 0.05 µmol/L) Test	Methotrexate (~ 0.50 µmol/L) Serum Control	Methotrexate (~ 0.50 µmol/L) Test (% Control)
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (94.0)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (106.6)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (108.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.1)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.6)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.0)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (99.0)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (96.3)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (100.3)

Specificity
Methotrexate's metabolites, folate analogs and other compounds having structural similarity were tested to determine whether these compounds affect the quantitation of methotrexate concentrations using the ARK Methotrexate Assay. High levels of these compounds were spiked into serum pools containing no methotrexate, 0.05 µmol/L or 0.50 µmol/L of methotrexate. The samples were analyzed and the methotrexate concentrations of samples containing interferent were compared to a serum control.

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite
After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 2 to 48 hours after HDMTX administration.[15,27,28,32,34-36]

Crossreactivity by 7-hydroxymethotrexate in the measurement of methotrexate was determined for the ARK Methotrexate Assay by testing paired samples containing (1) both 0.05 µmol/L methotrexate and 5 µmol/L 7-hydroxymethotrexate and (2) both 0.50 µmol/L methotrexate and 50 µmol/L 7-hydroxymethotrexate in human serum.

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.

Crossreactivity to 2,4-Diamino-N¹⁰-methylpteroic acid (DAMPA)
As a minor metabolite of methotrexate, DAMPA is not expected to circulate at concentrations that would interfere in measurement of methotrexate.[33] However, following glucarpidase rescue therapy, the serum concentration of DAMPA can be substantial.[13,14] The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64-fold to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact
The ARK Methotrexate Assay crossreacts slightly with trimetrexate and trimethoprim, however these drugs may be contraindicated for MTX cancer treatment due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

Compound	Methotrexate Absent: Tested (µmol/L)	MTX (µmol/L)	Cross Reactivity (%)	Methotrexate Present 0.05 µmol/L: MTX (µmol/L)	Cross Reactivity (%)	Methotrexate Present 0.50 µmol/L: MTX (µmol/L)	Cross Reactivity (%)
Trimetrexate	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.18	0.12	0.99	0.85

Document Control TECHNICAL Released Roche/Hitachi 917 Systems 2015-04-15 Revision History 02.2 2016-08-08

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Calibrator*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Calibrator must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CALIBRATOR	Calibrator

1 NAME

ARK™ *Methotrexate Calibrator*

2 INTENDED USE

ARK Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Calibrator is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description		Quantity/Volume
5026-0002-00	**ARK Methotrexate Calibrators* Methotrexate, buffer, bovine serum albumin, and preservatives**		Dropper vials
	A	0.00 µmol/L	1 X 2 mL
	B	0.05 µmol/L	1 X 2 mL
	C	0.15 µmol/L	1 X 2 mL
	D	0.25 µmol/L	1 X 2 mL
	E	0.50 µmol/L	1 X 2 mL
	F	1.20 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0227, 0.0682, 0.1136, 0.2272, and 0.5453 µg/mL for Calibrators B to F respectively.

4 STANDARDIZATION

There is no internationally recognized standard for Methotrexate. ARK Methotrexate Calibrators are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate.

5 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.
- Do not mix calibrators from different lot numbers.
- Use each lot as a set.

6 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Calibrators are ready to use. Mix each level by gentle inversion before dispensing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

7 PROCEDURE

Calibration

Perform a full calibration (6- point) procedure; test calibrators in duplicate. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

8 LIMITATIONS OF PROCEDURE

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

9 TRADEMARKS

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Calibrator Kit REF 5026-0002-00

ARK Diagnostics, Inc.
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Printed in USA
Revised August 2012
1600-0214-00 Rev 03

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Control*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

1 NAME

ARK™ *Methotrexate Control*

2 INTENDED USE

ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Control is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description	Quality Control
5026-0003-00 Complete Set	**ARK Methotrexate Control* (2 mL)** Methotrexate, buffer, bovine serum albumin, and preservatives (nominal level)	Expected Range (µmol/L)**
5026-0003-01 Calibration Range Controls[1]	LOW (0.07 µmol/L)	0.05 – 0.09
	MID (0.40 µmol/L)	0.30 – 0.50
	HIGH (0.80 µmol/L)	0.60 – 1.00
5026-0003-02 High Range Controls[2]	5 µmol/L	3.75 – 6.25
	50 µmol/L	37.50 – 62.50
	500 µmol/L	375.00 – 625.00

* To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0318, 0.1818, 0.3636, 2.272, 22.72, and 227.2 µg/mL respectively.

**The ARK Methotrexate Control is a precision control. Each laboratory should establish the mean value for each control level and its own ranges for each new lot of controls.

Each laboratory should establish its own ranges for each new lot of controls.

[1] Calibration range controls may be obtained separately as a set, REF 5026-0003-01.
[2] High range controls may be obtained separately as a set, REF 5026-0003-02.

4 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.
- Do not mix controls from different lot numbers.
- Use each lot as a set.

5 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Controls are ready to use. Mix each level by gentle inversion before dispensing.
- High range controls must be diluted prior to testing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

6 LIMITATIONS OF PROCEDURE

All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

7 TRADEMARKS

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Control Kit REF 5026-0003-00

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Printed in USA
Revised August 2012
1600-0215-00 Rev 03

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Dilution Buffer*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

1 NAME

ARK™ *Methotrexate Dilution Buffer*

2 INTENDED USE

ARK Methotrexate Dilution Buffer is intended for dilution of specimens containing high concentrations of methotrexate for the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Dilution Buffer is comprised of a synthetic protein matrix. Its composition is equivalent to Calibrator A (zero), ARK Methotrexate Calibrator, REF 5026-0002-00.

REF	Product Description	Quantity/Volume
5026-0004-00	**ARK Methotrexate Dilution Buffer** Buffer, bovine serum albumin, and preservatives	1 X 25 mL

4 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.

5 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Dilution Buffer is ready to use. Mix by gentle inversion before dispensing.
- Store at 2-8°C. Use prior to the expiration date. Once opened, store at 2-8°C for 12 months within the expiration date.

6 PROCEDURE – MANUAL DILUTION PROTOCOL

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L) are assayed by dilution of the specimen and controls into the measurement range.

Manually dilute the high specimen or high range control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

Sample Volume		Dilution Matrix Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor. To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

7 TRADEMARKS

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Printed in USA
Revised August 2012
1600-0216-00 Rev 03

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Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
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October 31, 2011

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Document No: k111904
Re: k111904
Received: July 5, 2011

Categorization Notification

Regulations codified at 42 CFR 493.17 et. seq., implementing the Clinical Laboratory Improvement Amendments of 1988, require the Secretary to provide for the categorization of specific clinical laboratory test systems by the level of complexity. Based upon these regulations, the following commercially marketed test system or assay for the analyte is categorized below:

Test System/Analyte (s) : (SEE ATTACHMENT)

This complexity categorization is effective as of the date of this notification and will be reported on FDA's home page http://www.fda.gov/cdrh/clia. This categorization information may be provided to the user of the commercially marketed test system or assay as specified for the analyte indicated. It will also be announced in a Federal Register Notice, which will provide opportunity for comment on the decision. FDA reserves the right to reevaluate and recategorize this test based upon the comments received in response to the Federal Register Notice.

If you change the test system name or your company's name or if a distributor's name replaces your name, you must request another categorization by sending in the revised labeling along with a letter to FDA referencing the document number above.

If you have any questions regarding this complexity categorization, please contact Zhihao Peter Qiu at 301-796-6655.

Sincerely yours,

Alberto Gutierrez, Ph.D.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ATTACHMENT

Document Number : k111904

Test System: Roche Diagnostics Hitachi 917 (ARK Diagnostics Methotrexate Assay)
Analyte : Methotrexate
Complexity : MODERATE

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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CLIA Routing Slip

Document No : k111904

Re : k111904

Division: DCTD
Branch: CTXB

Applicant: Ark Diagnostics,Inc
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Trade Name: Ark methotrexate assay, ark methotrexate calibrator, ark methotrexate control, ark methotrexate control (calibration ran

DMC Date Received: July 5, 2011
Division Date Received: July 8, 2011

Categorization Information

CLIA Reviewer: Kellie Kelm [KBK]
Date Review Completed: October 17, 2011

Date Branch Concurred: 10-21-2011 RCL
Date Coordinator Concurred: OCT 31 2011 KCB
Effective Date:

Test Systems/Analytes/Grading

(See Attachment)

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ATTACHMENT

Document Number : k111904

Test System: Roche Diagnostics Hitachi 917 (ARK Diagnostics Methotrexate Assay)
Analyte : Methotrexate
Complexity : MODERATE [11]
Knowledge [2]; Training and Experience [2]; Reagents Preparation [1];
Operational Steps [1]; Quality Control [1];
Troubleshooting and Maintenance [2]; Interpretation and Judgment [2]
Rationale : CHEM-001

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ARK™ *Methotrexate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2

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Reagent Kit REF 5026-0001-00

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Name

ARK™ *Methotrexate Assay*

1 Intended Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

2 Summary and Explanation of the Test

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino]benzoyl]-L-glutamic acid], formerly Amethopterin, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1-3] Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Guidelines for methotrexate therapy with leucovorin rescue should be consulted.[1] Intermediate to high doses of methotrexate (approximately 35 mg/m^2 - 12 g/m^2) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[4-8]

3 Principles of the Procedure

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

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4 Reagents

REF	Product Description	Quantity/Volume
5026-0001-00	**ARK Methotrexate Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative, and stabilizers	**1 X 16 mL**
	Reagent R2 – Enzyme methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative, and stabilizers	**1 X 8 mL**

Reagent Handling and Storage

ARK Methotrexate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

5 Warnings and Precautions

- For In Vitro Diagnostic Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 Specimen Collection and Preparation for Analysis

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice.
- The sampling time of methotrexate will be dependent on dose, duration of infusion, and clinical status of the patient. Consult specific treatment protocols for sampling time.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
    - Sodium heparin
    - Lithium heparin
    - Potassium EDTA
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

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- Clarified specimens may be stored up to two weeks at 2 to 8°C. If testing will be delayed, specimens may be stored frozen (≤ -10°C) prior to being tested. Care should be taken to limit the number of freeze-thaw cycles. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C.
- **Handle all patient specimens as if they were potentially infectious.**

8 Procedure

Materials Provided
ARK Methotrexate Assay – REF 5026-0001-00

Materials Required – Provided Separately
ARK Methotrexate Calibrator – REF 5026-0002-00
Quality Controls – ARK Methotrexate Control – REF 5026-0003-00
ARK Methotrexate Dilution Buffer – REF 5026-0004-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Calibration
Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E, and F; test calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

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Quality Control (QC)
Laboratories should establish QC procedures for the ARK Methotrexate Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol
The range of the ARK Methotrexate calibration curve is 0.00 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the range of the calibration curve.

Manually dilute the high specimen or control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

	Sample Volume	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor.

9 Results

To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

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10 Limitations of Procedure

This assay is designed for use with serum or plasma only; refer to the sections Specimen Collection and Preparation for Analysis. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variability. See the section Expected Values below.

As with all analyte determinations, the methotrexate value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

IMPORTANT: Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay. These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid (DAMPA)[10-12] that result from metabolism of methotrexate by glucarpidase. DAMPA crossreacts with the methotrexate antibody used in this assay, and may continue to circulate for at least five to seven days before accurate measurements of serum methotrexate may return.[13] Oncologists on the clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA that would confuse the efforts of glucarpidase therapy.[13] While glucarpidase is well tolerated and rapidly reduces circulating MTX, delayed renal elimination of MTX can still be a problem for adult and elderly patients.[14]

11 Expected Values

Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors.[1,3] While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example),[15] concentrations may exceed 1000 µmol/L[16] with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.[17] For treatment of osteosarcoma,[16] the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, less than 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin in accordance with algorithms until the MTX level is <0.1 µmol/L. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.[1, 9] Some centers follow ≤ 0.10 µmol/L.[16, 18]

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From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.[1, 9, 19]

Clinical Situation	Laboratory Findings	
	Methotrexate Level (µmol/L)	Hours after administration
Normal Methotrexate Elimination	~10 ~1 <0.2	24 48 72
Delayed Late Methotrexate Elimination	>0.2 >0.05	72 96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50 ≥5 OR ≥100% increase in serum creatinine	24 48 24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs, [14, 19] for example vancomycin.[20] Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.[21-28]

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite,[15, 27] its molar ratio to methotrexate of up to approximately 100-fold,[29] and relative insolubility versus the parent drug,[14, 19] possible nephrotoxicity due to precipitation of the metabolite in renal tubules[29] may delay elimination of methotrexate itself.

Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.[14] Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.[13]

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12 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche/Hitachi 917 System.

Limit of Quantitation (LoQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

Measurement Range

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the measurement range.

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Recovery

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

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Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (monoclonal FPIA)[30].

Methotrexate concentrations by FPIA ranged 0.04 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.04 to 1500 µmol/L. Results of the Passing-Bablok[31] regression analysis for the study are shown below (with 95% confidence limits) for 102 specimens within the measurement range as well as for all 147 specimens including those above the measurement range requiring dilution.

Parameter	Range 0.04 to 1.19 µM		Range 0.04 to 1440 µM	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.00 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.978	(0.968 to 0.985)	0.998	(0.997 to 0.998)
Number of Samples	102	na	147	na

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.1 µmol/L, SD ≤0.01 at ≤0.1 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		Methotrexate (~ 0.50 µmol/L)	
Interfering Substance	Interferent Concentration	Serum Control	Test	Serum Control	Test (% Control)
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity

Methotrexate's metabolites, folate analogs and other compounds having structural similarity were tested to determine whether these compounds affect the quantitation of methotrexate concentrations using the ARK Methotrexate Assay. High levels of these compounds were spiked into serum pools containing no methotrexate, 0.05 µmol/L or 0.50 µmol/L of methotrexate. The samples were analyzed and the methotrexate concentrations of samples containing interferent were compared to a serum control.

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Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 12 to 48 hours after HDMTX administration.[15, 27, 29, 32, 34-35]

Crossreactivity by 7-hydroxymethotrexate in the measurement of methotrexate was determined for the ARK Methotrexate Assay by testing paired samples containing (1) both 0.05 µmol/L methotrexate and 5 µmol/L 7-hydroxymethotrexate and (2) both 0.50 µmol/L methotrexate and 50 µmol/L 7-hydroxymethotrexate in human serum.

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.

Crossreactivity to 2,4-Diamino-N^{10}-methylpteroic acid (DAMPA)

As a minor metabolite of methotrexate, DAMPA is not expected to circulate at concentrations that would interfere in measurement of methotrexate.[33] However, following glucarpidase rescue therapy, the serum concentration of DAMPA can be substantial.[13, 14] The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for MTX cancer treatment due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

		Methotrexate Absent		Methotrexate Present 0.05 µmol/L		Methotrexate Present 0.50 µmol/L	
Compound	Tested (µmol/L)	MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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14 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised September 2011
1600-0213-00 Rev 03

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Services
Food and Drug Administration

Memorandum

Date:

From: DMC (HFZ-401)

Subject: Premarket Notification Number(s): K111904/A1

To: Division Director: TX / DCTD

The attached information has been received by the 510(k) DMC on the above referenced 510(k) submission(s). Since a final decision has been rendered, this record is officially closed.

Please review the attached document and return it to the DMC, with one of the statements checked below.

_____ Information does not change the status of the 510(k); no other action required by the DMC; please add to image file. (Prepare K-25) THIS DOES NOT APPLY TO TRANSFER OF OWNERSHIP. PLEASE BRING ANY TRANSFER OF OWNERSHIP TO POS.

_____ Additional information requires a new 510(k); however, the information submitted is incomplete; (Notify company to submit a new 510(k); [Prepare the K30 Letter on the LAN]

✓ No response necessary (e.g., hard copy of fax for the truthful and accuracy statement, 510(k) statement, change of address, phone number, or fax number).

CLIA CATEGORIZATION refers to laboratory test system devices reviewed by the Division of Clinical Laboratory Devices (HFZ-440

_____ Information requires a **CLIA CATEGORIZATION**; the complexity may remain the same as the original 510(k) or may change as a result of the additional information (Prepare a CAT letter)

_____ Additional information requires a **CLIA CATEGORIZATION**; however, the information submitted is incomplete; (call or fax firm)

✓ No response necessary "Final" labeling submitted by Sponsor.

This information should be returned to the DMC within 10 working days from the date of this Memorandum.

Reviewed by: [signature]

Date: 11/14/2011

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

K111904/A1

ARK Diagnostics, Inc.
Quality health through drug management

November 7, 2011

FDA/CDRH/DCC
NOV 8 2011
RECEIVED

Document Mail Center (WO66-0609)
OIVD/CDRH/FDA
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

RE: 510(K) Premarket Notification K111904, Finished labeling

ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, and ARK™ Methotrexate Control, ARK™ Methotrexate Dilution Buffer

Dear FDA Staff,

ARK Diagnostics submits a copy of its finished labeling according to Doc. No. 0743-0026-00 Rev 03. This document contains copies of container labels and package inserts for the ARK Methotrexate Assay (Model No. 5026-0001-00), ARK Methotrexate Calibrator (Model No. 5026-0002-00), ARK Methotrexate Control (Model No. 5026-0003-00) and ARK Methotrexate Dilution Buffer (Model No. 5026-0004-00).

Please feel free to contact me if you have any questions, or require additional information, and please note my contact information below.

Sincerely,

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.

Tel (408) 747-0708
FAX (408) 747-0783
e-mail: ken@ark-tdm.com

K-22

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Label Proof – Methotrexate Products

1. PURPOSE

This document comprises the label proofs for comparison of raw material stock labels for the Methotrexate assay products, reagents, calibrators, controls and dilution buffer. The label proofs are actual, approved pre-printed stock labels. It also comprises appended copies of the current approved version of package inserts to facilitate material inspection.

2. SCOPE

The process guideline applies to the inspection of raw material stock labels and package inserts for Methotrexate assay products at ARK Diagnostics, Inc (ARK).

3. REFERENCES

0740-0026-01 – Purchase Requisition for Methotrexate Package Inserts
0743-0001-00 – Receiving and Inspection
0743-0001-01 – Receiving Purchase Order - Inventoried Item
0900-0003-38 – Product Label Printing In-House
0900-0004-23 – Specification, ARK Methotrexate Package Inserts
0900-0004-24 – Label Proofs – Methotrexate Assay

4. RESPONSIBILITIES

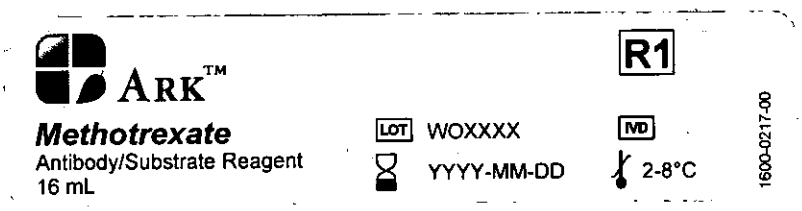
OPERATIONS and QA are responsible for maintaining this guideline.

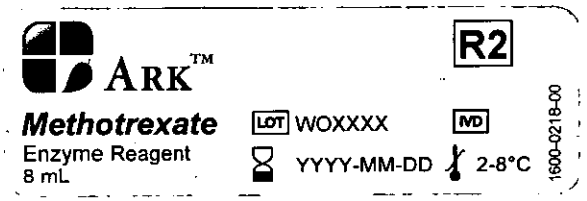
5. PROCEDURE

5.1. Container Labels for Methotrexate assay products are produced according to Doc. No. 0900-0003-38 – Product Label Printing In-House. Compare the label for its respective product.

5.2. Consult this label proof for each label part number. Verify the lot number and expiration date of produced labels according to the Production Order and printing procedure. This label proof is comprised of labels with generic lot number **WOXXXX** and expiration date **YYYY/MM/DD**.

5.3. Bottle Labels for Methotrexate Reagents





Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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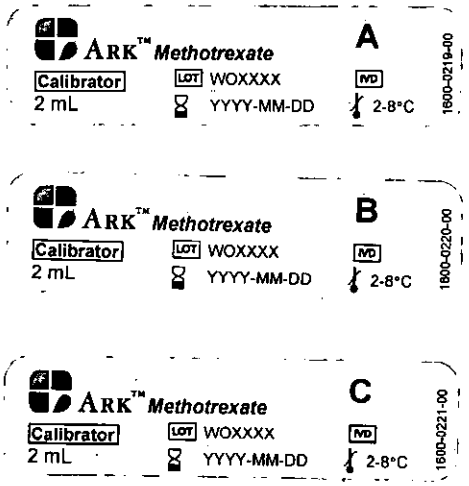
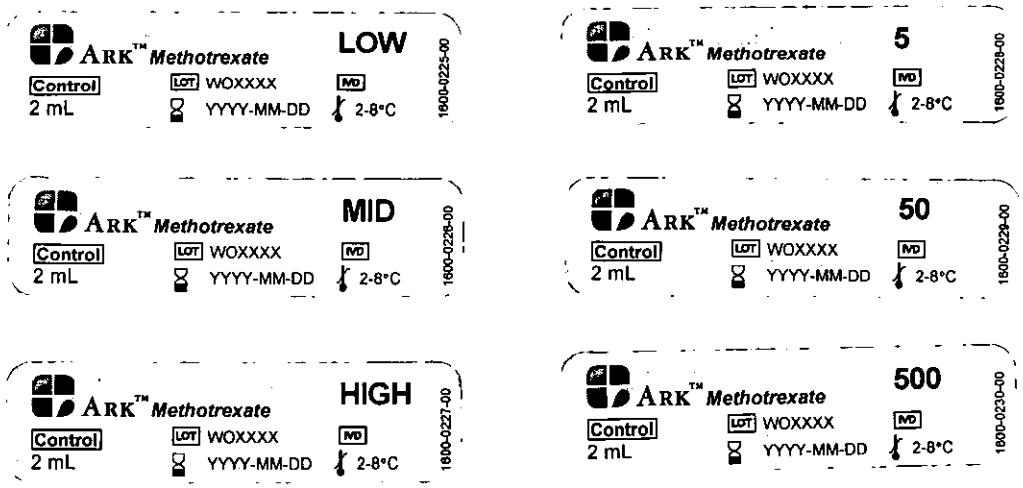
Label Proof – Methotrexate Products

5.4. Bottle Labels for Methotrexate Calibrators



5.5. Bottle Labels for Methotrexate Controls



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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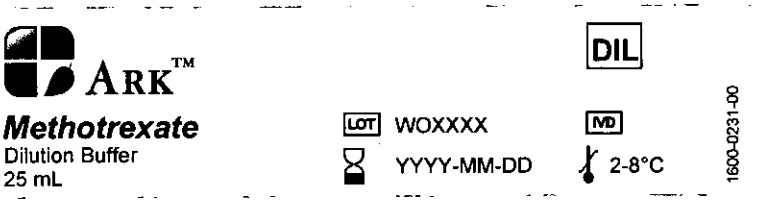
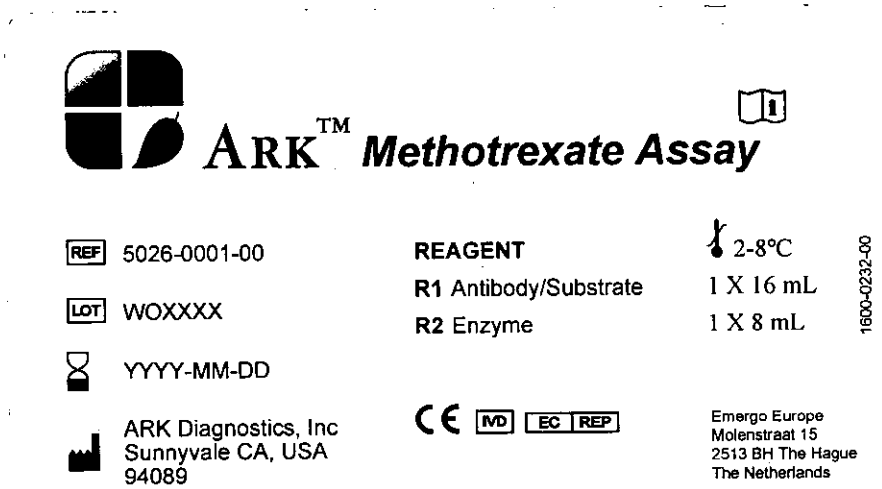
Label Proof – Methotrexate Products

5.6. Bottle Label for Methotrexate Dilution Buffer



5.7. Kit Label for Methotrexate Reagents



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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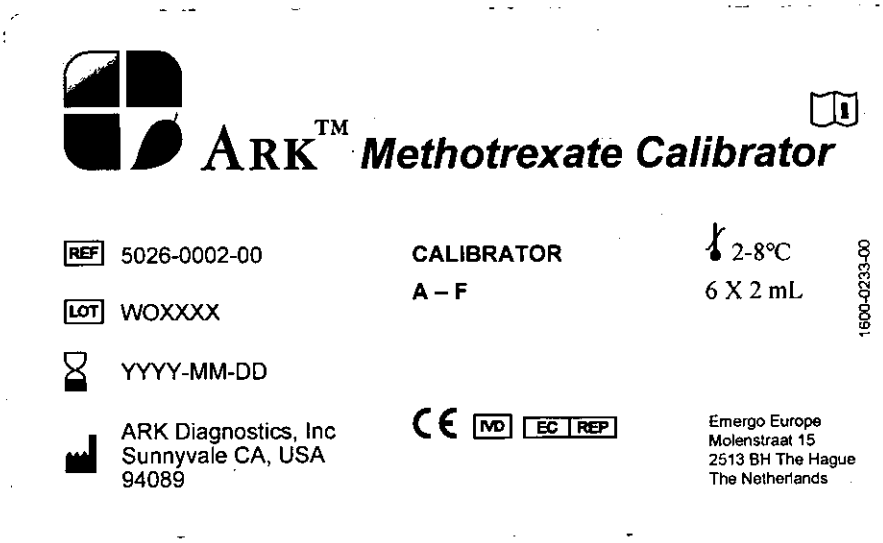
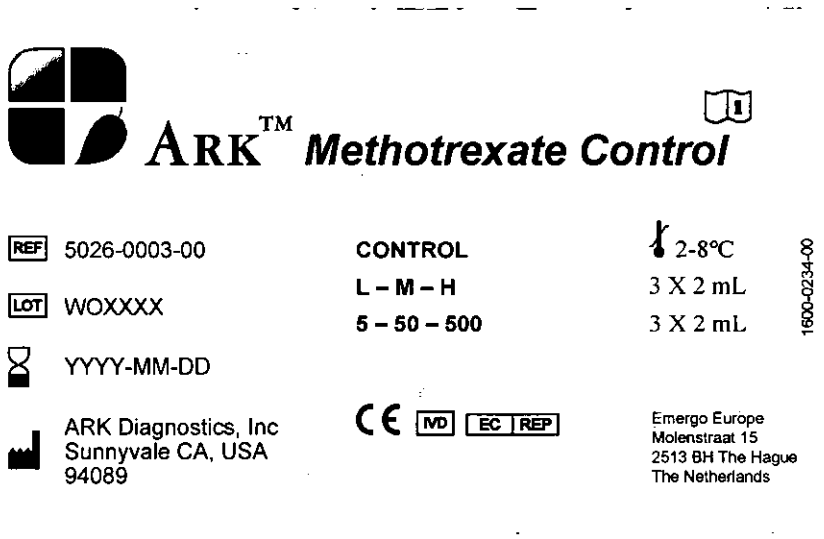
Label Proof – Methotrexate Products

5.8. Kit Label for Methotrexate Calibrators



5.9. Kit Label for Methotrexate Controls [complete set]



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Label Proof – Methotrexate Products

5.10. Kit Label for Methotrexate Controls [Calibration Range set]

5.11. Kit Label for Methotrexate Controls [High Range set]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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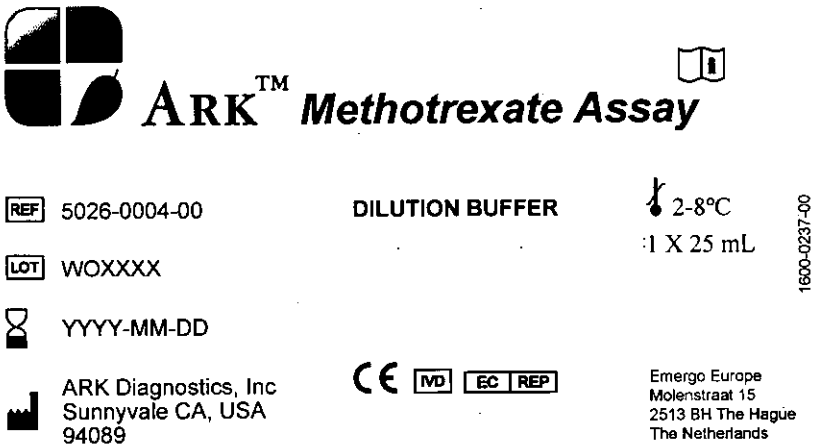
Label Proof – Methotrexate Products

5.12. Kit Label for Methotrexate Dilution Buffer



5.13. Appended copies of package inserts:

- 1600-0213-00 – October 2011, Rev03, Methotrexate Assay
- 1600-0214-00 – October 2011, Rev02, Methotrexate Calibrator
- 1600-0215-00 – October 2011, Rev02, Methotrexate Control
- 1600-0216-00 – October 2011, Rev02, Methotrexate Dilution Buffer

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

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Tel: (31) (0) 70 345-8570
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KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 / R2	Reagent 1/ Reagent 2

1 NAME

ARK™ *Methotrexate Assay*

2 INTENDED USE

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

3 SUMMARY AND EXPLANATION OF THE TEST

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino]benzoyl]-L-glutamic acid], formerly Amethopterin, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1-3] Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Guidelines for methotrexate therapy with leucovorin rescue should be consulted.[1] Intermediate to high doses of methotrexate (approximately 35 mg/m² - 12 g/m²) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[4-8]

4 PRINCIPLES OF THE PROCEDURE

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

5 REAGENT

REF	Product Description	Quantity/Volume
5026-0001-00	**ARK Methotrexate Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative, and stabilizers	1 X 16 mL
	Reagent R2 – Enzyme methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative, and stabilizers	1 X 8 mL

Reagent Handling and Storage

ARK Methotrexate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

6 WARNINGS AND PRECAUTIONS

- For **In Vitro Diagnostic** Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice.
- The sampling time of methotrexate will be dependent on dose, duration of infusion, and clinical status of the patient. Consult specific treatment protocols for sampling time.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
  - Sodium heparin
  - Lithium heparin
  - Potassium EDTA
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.
- Clarified specimens may be stored up to two weeks at 2 to 8°C. If testing will be delayed, specimens may be stored frozen (≤ -10°C) prior to being tested. Care should be taken to limit the number of freeze-thaw cycles. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C.
- **Handle all patient specimens as if they were potentially infectious.**

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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Calibrator*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Calibrator must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.
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Sunnyvale, CA 94089 USA
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The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CALIBRATOR	Calibrator

1 NAME

ARK™ *Methotrexate Calibrator*

2 INTENDED USE

ARK Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Calibrator is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description		Quantity/Volume
5026-0002-00	**ARK Methotrexate Calibrators* Methotrexate, buffer, bovine serum albumin, and preservatives**		Dropper vials
	A	0.00 µmol/L	1 X 2 mL
	B	0.05 µmol/L	1 X 2 mL
	C	0.15 µmol/L	1 X 2 mL
	D	0.25 µmol/L	1 X 2 mL
	E	0.50 µmol/L	1 X 2 mL
	F	1.20 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0227, 0.0682, 0.1136, 0.2272, and 0.5453 µg/mL for Calibrators B to F respectively.

4 STANDARDIZATION

There is no internationally recognized standard for Methotrexate. ARK Methotrexate Calibrators are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate.

5 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.
- Do not mix calibrators from different lot numbers.
- Use each lot as a set.

6 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Calibrators are ready to use. Mix each level by gentle inversion before dispensing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

7 PROCEDURE

Calibration

Perform a full calibration (6- point) procedure; test calibrators in duplicate. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate

- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

8 LIMITATIONS OF PROCEDURE

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

9 TRADEMARKS

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

© 2011, ARK Diagnostics, Inc.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0214-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Control*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

1 NAME

ARK™ *Methotrexate Control*

2 INTENDED USE

ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Control is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description	Quality Control
5026-0003-00 Complete Set	**ARK Methotrexate Control* (2 mL)** Methotrexate, buffer, bovine serum albumin, and preservatives (nominal level)	Expected Range (µmol/L)**
5026-0003-01 Calibration Range Controls[1]	LOW (0.07 µmol/L)	0.05 – 0.09
	MID (0.40 µmol/L)	0.30 – 0.50
	HIGH (0.80 µmol/L)	0.60 – 1.00
5026-0003-02 High Range Controls[2]	5 µmol/L	3.75 – 6.25
	50 µmol/L	37.50 – 62.50
	500 µmol/L	375.00 – 625.00

* To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0318, 0.1818, 0.3636, 2.272, 22.72, and 227.2 µg/mL respectively.

**The ARK Methotrexate Control is a precision control. Each laboratory should establish the mean value for each control level and its own ranges for each new lot of controls.

Each laboratory should establish its own ranges for each new lot of controls.

[1] Calibration range controls may be obtained separately as a set, REF 5026-0003-01.
[2] High range controls may be obtained separately as a set, REF 5026-0003-02.

4 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.
- Do not mix controls from different lot numbers.
- Use each lot as a set.

5 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Controls are ready to use. Mix each level by gentle inversion before dispensing.
- High range controls must be diluted prior to testing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

6 LIMITATIONS OF PROCEDURE

All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

7 TRADEMARKS

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Control Kit REF 5026-0003-00

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0215-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

ARK™ *Methotrexate Dilution Buffer*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

CUSTOMER SERVICE

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

KEY TO SYMBOLS USED

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

1 NAME

ARK™ *Methotrexate Dilution Buffer*

2 INTENDED USE

ARK Methotrexate Dilution Buffer is intended for dilution of specimens containing high concentrations of methotrexate for the ARK Methotrexate Assay, REF 5026-0001-00.

3 CONTENT

ARK Methotrexate Dilution Buffer is comprised of a synthetic protein matrix. Its composition is equivalent to Calibrator A (zero), ARK Methotrexate Calibrator, REF 5026-0002-00.

REF	Product Description	Quantity/Volume
5026-0004-00	**ARK Methotrexate Dilution Buffer** Buffer, bovine serum albumin, and preservatives	1 X 25 mL

4 WARNINGS AND PRECAUTIONS

- For ***In Vitro Diagnostic*** Use.

5 INSTRUCTIONS FOR USE

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Dilution Buffer is ready to use. Mix by gentle inversion before dispensing.
- Store at 2-8°C. Use prior to the expiration date. Once opened, store at 2-8°C for 12 months within the expiration date.

6 PROCEDURE – MANUAL DILUTION PROTOCOL

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L) are assayed by dilution of the specimen and controls into the measurement range.

Manually dilute the high specimen or high range control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

Sample Volume		Dilution Matrix Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Specimen} + \text{Volume of Dilution Buffer})}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor. To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

7 TRADEMARKS

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

© 2011, ARK Diagnostics, Inc.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0216-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records Processed under FOIA Request # 2016-8894; Released by CDRH on 02-22-2016

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

OCT 18 2011

ARK Diagnostics, Inc
c/o Kenneth C. Kasper, PhD
1190 Bordeaux Dr.
Sunnyvale, CA 94089

Re: k111904
Trade/Device Name: ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, ARK™ Methotrexate Control
Regulatory Class: Unclassified, 510(k) required
Product Code: LAO, DLJ, LAS
Dated: September 7, 2011
Received: September 8, 2011

Dear Dr. Kasper:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at (301) 796-5760. For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-5680 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Indication for Use

510(K) Number (if known): K111904

Device Name:

ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control

Indications for Use:

The ARK™ Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of Methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK™ Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay.

The ARK™ Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Prescription Use ___X___ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) 111904

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

August 24, 2011

ARK DIAGNOSTICS,INC
1190 BORDEAUX DRIVE
SUNNYVALE, CALIFORNIA 94089
ATTN: KENNETH C. KASPER

510k Number: K111904
Product: ARK METHOTREXATE ASSAY, ARK ME

Extended Until: 01/12/2012

Based on your recent request, an extension of time has been granted for you to submit the additional information we requested.

If the additional information (AI) is not received by the "Extended Until" date shown above, your premarket notification will be considered withdrawn (21 CFR 807.87(l)). If the submitter does submit a written request for an extension, FDA will permit the 510(k) to remain on hold for up to a maximum of 180 days from the date of the AI request.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely yours,

Marjorie Shulman
Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.
Quality Health Through Drug Management

August 18, 2011

Marjorie Shulman
Consumer Safety Officer, Premarket Notification Section
Food and Drug Administration
ODE/OIVD/CDRH
10903 New Hampshire Avenue
Document Mail Center - WO66-G609
Silver Spring, MD 20993-0002

FDA CDRH DMC
AUG 19 2011
Received

RE: 510(K) Premarket Notification K111904;

ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, ARK™ Methotrexate Control, and ARK™ Methotrexate Dilution Buffer

Request for extension of 180 days

Dear Ms. Shulman,

ARK Diagnostics, Inc. requests an extension of 180 days for the review of its bundled submission K111904 for the ARK™ Methotrexate Assay, Calibrators, Controls and Dilution Buffer. ARK has received the Hold Letter from FDA dated August 17, 2011 by e-mail from the scientific reviewer, Dr. Kellie Kelm. Issues raised are being clarified with the scientific reviewer. To the extent possible ARK Diagnostics will complete the additional information requested promptly.

Please feel free to contact me if you have any questions, or require additional information, and please note my contact information below.

Sincerely,

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.

Tel (408) 747-0708
FAX (408) 747-0783
e-mail: ken@ark-tdm.com

121

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

August 18, 2011

ARK DIAGNOSTICS,INC
1190 BORDEAUX DRIVE
SUNNYVALE, CALIFORNIA 94089
ATTN: KENNETH C. KASPER

510k Number: K111904
Product: ARK METHOTREXATE ASSAY, ARK ME

We are holding your above-referenced Premarket Notification (510(k)) for 30 days pending receipt of the additional information that was requested by the Office of Device Evaluation. Please remember that all correspondence concerning your submission MUST cite your 510(k) number and be sent in duplicate to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm.

The deficiencies identified represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed. In developing the deficiencies, we carefully considered the statutory criteria as defined in Section 513(i) of the Federal Food, Drug, and Cosmetic Act for determining substantial equivalence of your device. We also considered the burden that may be incurred in your attempt to respond to the deficiencies. We believe that we have considered the least burdensome approach to resolving these issues. If, however, you believe that information is being requested that is not relevant to the regulatory decision or that there is a less burdensome way to resolve the issues, you should follow the procedures outlined in the "A Suggested Approach to Resolving Least Burdensome Issues" document. It is available on our Center web page at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/Overview/MedicalDeviceProvisionsofFDAModernizationAct/ucm136685.htm.

If after 30 days the additional information (AI), or a request for an extension of time, is not received, we will discontinue review of your submission and proceed to delete your file from our review system (21 CFR 807.87(l)). Please note our guidance document entitled, "Guidance for Industry and FDA Staff, FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". If the submitter does submit a written request for an extension, FDA will permit the 510(k) to remain on hold for up to a maximum of 180 days from the date of the AI request. The purpose of this document is to assist agency staff and the device industry in understanding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee and Modernization Act. You may review this document at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm. Pursuant to 21 CFR 20.29, a copy of your 510(k) submission will remain in the Office of Device Evaluation. If you then wish to resubmit this 510(k) notification, a new number will be assigned and your submission will be considered a new premarket notification submission.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-6894; Released by CDRH on 02-22-2016

Please remember that the Safe Medical Devices Act of 1990 states that you may not place this device into commercial distribution until you receive a decision letter from FDA allowing you to do so.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely yours,

Marjorie Shulman
Consumer Safety Officer
Premarket Notification Section
Office of Device Evaluation
Center for Devices and Radiological Health

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

July 07, 2011

ARK DIAGNOSTICS,INC
1190 BORDEAUX DRIVE
SUNNYVALE, CALIFORNIA 94089
ATTN: KENNETH C. KASPER

510k Number: K111904
Received: 7/5/2011
Product: ARK METHOTREXATE ASSAY, ARK ME

The Center for Devices and Radiological Health (CDRH), Office of Device Evaluation (ODE), has received the Premarket Notification you submitted in accordance with Section 510(k) of the Federal Food, Drug, and Cosmetic Act(Act) for the above referenced product. We have assigned your submission a unique 510(k) number that is cited above. Please refer prominently to this 510(k) number in any future correspondence that relates to this submission. We will notify you when the processing of your premarket notification has been completed or if any additional information is required. YOU MAY NOT PLACE THIS DEVICE INTO COMMERCIAL DISTRIBUTION UNTIL YOU RECEIVE A LETTER FROM FDA ALLOWING YOU TO DO SO.

On May 21, 2004, FDA issued a Guidance for Industry and FDA Staff entitled, "FDA and Industry Actions on Premarket Notification (510(k)) Submissions: Effect on FDA Review Clock and Performance Assessment". The purpose of this document is to assist agency staff and the device industry in understanding how various FDA and industry actions that may be taken on 510(k)s should affect the review clock for purposes of meeting the Medical Device User Fee and Modernization Act. Please review this document at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089735.htm.

In future premarket submissions, we encourage you to provide an electronic copy of your submission. By doing so, you will save FDA resources and may help reviewers navigate through longer documents more easily. Under CDRH's eCopy Program, you may replace one paper copy of any premarket submission (e.g., 510(k), IDE, PMA, HDE) with an electronic copy. For more information about the program, including the formatting requirements, please see http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/ucm134508.htm.

We remind you that Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) amended the PHS Act by adding new section 402(j) (42 U.S.C. § 282(j)), which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices. Section 402(j) requires that a certification form (http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3674.pdf) accompany 510(k)/HDE/PMA submissions. The agency has issued a draft guidance titled: "Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of The Food and Drug Administration

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016
Amendments Act of 2007" (http://www.fda.gov/oc/initiatives/fdaaa/guidance_certifications.html). According to the draft guidance, 510(k) submissions that do not contain clinical data do not need the certification form.

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) requires the categorization of commercially marketed test systems by level of complexity. If your device is a test system that requires categorization you will be notified of your complexity as an enclosure with any clearance letter.

Please remember that all correspondence concerning your submission MUST be sent to the Document Mail Center (DMC) at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review". Please refer to this guidance for information on current fax and e-mail practices at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm.

Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

You should be familiar with the regulatory requirements for medical device available at Device Advice http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm". If you have other procedural questions, or want information on how to check on the status of your submission, please contact DSMICA at (301)796-7100 or its toll-free number (800)638-2041, or at their Internet address http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/default.htm or the 510k staff at (301)796-5640 .

Sincerely,

510(k) Staff

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Submitter:
Kenneth C. Kasper
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA, 94089, US
Phone: (408) 747-0708
Fax: (408) 747-0783

Secondary Contact:
Johnny Valdez
President
ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA, 94089, US
Phone: (408) 747-0706
Fax: (408) 747-0783

July 1, 2011

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Attn: eSubmitter Team
Document Mail Center - WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

Dear Sir or Madam:

This submission dated July 1, 2011, is filed pursuant to the In Vitro Diagnostic Device Evaluation and Safety program's Electronic Review (eReview).

The submission is a(n) **510(k)**, **Original Submission**, **Traditional** referring to the product, Homogeneous Enzyme Immunoassay also known as trade name(s): ARK Methotrexate Assay, ARK Methotrexate Calibrator, ARK Methotrexate Control, ARK Methotrexate Control (Calibration Range), ARK Methotrexate Control (High Range), and ARK Methotrexate Dilution Buffer with model(s): 5026-0001-00, 5026-0002-00, 5026-0003-00, 5026-0003-01, 5026-0003-02, and 5026-0004-00. The classification panel for this submission is CLINICAL TOXICOLOGY.

Indications for Use

The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

The prior submissions that are related to this release are as follows: NA.
The devices to which substantial equivalence is claimed are as follows: K932615 Abbott TDx/TDxFLx Methotrexate II assay.

User Fee Payment ID: MD6056070956733

Truth and Accuracy Statement

To the best of my knowledge, the data and information submitted in this premarket notification are truthful and accurate, and no material fact has been omitted (as required by 21 CFR 807.87).

Sincerely,

Kenneth C. Kasper
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Submission Report

Section: Admin

1.0 Type of Submission

Introduction

Department of Health and Human Services
Food and Drug Administration

CDRH PreMarket Review Submission Cover Sheet

Form Approval
OMB No. 0910-0120

Note:	*Please be advised that: 1) under 21 CFR 807.7(1), we may request any additional information that is necessary to reach a determination regarding substantial equivalence and 2) supportive raw data may be provided as attachments to this submission.*
Information:	*Whenever possible, please enter descriptions in executive summary format because this information will be used to generate the FDA Decision summary. If additional information is needed, please attach the appropriate file.*

Section A	**Type of Submission**

Submission Type		*	510(k)
.	Submission Sub-Type	*	Original Submission
-	Submission Sub-Sub-Type	*	Traditional
Enter the original submission number.			
Is this a bundled submission?		*	Yes

User Fee Payment ID Number	*	MD6056070956733

MDUFMA Cover Sheet

Please attach the completed MDUFMA Cover Sheet.		*
File Attachment	MDUFMA Cover Sheet	

2.0 Contact Information

Section B	**Primary Contact (Submitter, Applicant, or Sponsor)**

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Primary Contact Information	*
Contact Information:	
Contact Name	Dr. Kenneth C. Kasper
Occupation Title	Executive Director, Quality and Regulatory Affairs
Email Address	ken@ark-tdm.com
Address	
Establishment Name	ARK Diagnostics, Inc.
Division Name	
Address	1190 Bordeaux Drive Sunnyvale, CA, 94089, US
Telephone Number	(408) 747-0708
Fax Number	(408) 747-0783

Section C	**Secondary Contact (Applicant Correspondent, US Agent, or Consultant)**

Secondary Contact Information	
Contact Information:	
Contact Name	Mr. Johnny Valdez
Occupation Title	President
Email Address	johnny@ark-tdm.com
Address	
Establishment Name	ARK Diagnostics, Inc.
Division Name	
Address	1190 Bordeaux Drive Sunnyvale, CA, 94089, US
Telephone Number	(408) 747-0706
Fax Number	(408) 747-0783

Who should be contacted for issues related to this submission? *	Primary Contact

Section D	**Manufacturing Location (Physical Location of the Manufacturing Plant)**

Manufacturing Location (Optional)	
Establishment Information:	
Establishment Name	ARK Diagnostics, Inc.
FDA Establishment Identifier (FEI)	
Central File Number (CFN)	
Registration Number	3005755244
Owner/Operator Number	10027663
D&B D-U-N-S Number	
Physical Location:	
Address	1190 Bordeaux Drive Sunnyvale, CA, 94089, US
Telephone Number	(408) 747-0700

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Fax Number	(408) 747-0783

3.0 Reason for Submission

Section D3	**Reason for Submission - 510(k)**

New Device	*	Yes
Additional or Expanded Indications		
Change in technology		
If additional information is required, attach file.		

Section E	**Additional Information on 510(k) submissions**

Information on Device to which substantial equivalence is claimed. 510(k) Number - Trade or Proprietary or Model Name		*
Item 1	K932615 Abbott TDx/TDxFLx Methotrexate II assay	

For this submission, are you including a 510(k) Statement or Summary?	*	510(k) Summary
Enter your 510(k) Summary or Statement.		*
The 510(k) Summary for the ARK Methotrexate Assay system is attached.		
File Attachment	510(k) Summary	

4.0 Product Information

Section F	**Product Information**

Common or classification name	*
Homogeneous Enzyme Immunoassay	

FDA Document Numbers of all prior related submissions (regardless of outcome)		*
Item 1	NA	

4.1 Trade, Proprietary, or Model Names

Item: 1

Trade, proprietary, or model name for this device	*	ARK Methotrexate Assay
Model number	*	5026-0001-00

Item: 2

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Trade, proprietary, or model name for this device	*	ARK Methotrexate Calbrator
Model number	*	5026-0002-00

Item: 3

Trade, proprietary, or model name for this device	*	ARK Methotrexate Control
Model number	*	5026-0003-00

Item: 4

Trade, proprietary, or model name for this device	*	ARK Methotrexate Control (Calibration Range)
Model number	*	5026-0003-01

Item: 5

Trade, proprietary, or model name for this device	*	ARK Methotrexate Control (High Range)
Model number	*	5026-0003-02

Item: 6

Trade, proprietary, or model name for this device	*	ARK Methotrexate Dilution Buffer
Model number	*	5026-0004-00

5.0 Product Classification

Section G1	**Product Classification**

Choose the product code for this submission.

Product Code	ENZYME IMMUNOASSAY, METHOTREXATE (LAO)
Device Class	UNCLASSSIFIED
Classification Panel	CLINICAL TOXICOLOGY
C.F.R. Section	-

Add any other product codes that are applicable to this submission.

Item	Product Code	Device Class	Classification Panel	C.F.R. Section
Item 1	CALIBRATORS, DRUG SPECIFIC (DLJ)	CLASS II	CLINICAL TOXICOLOGY	862.3200 - CLINICAL TOXICOLOGY CALIBRATOR.
Item 2	METHOTREXATE CONTROL MATERIALS (LAY)	CLASS I	CLINICAL TOXICOLOGY	862.3280 - CLINICAL TOXICOLOGY CONTROL MATERIAL.

If the product code(s) is/are unknown, please describe the classification of the product.

Class 2; ARK Methotrexate Dilution Buffer is intended for use with the ARK Methotrexate Assay. It is a diluent used to prepare specimens with concentrations of methotrexate that exceed the calibration range.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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If necessary, identify the secondary Classification Panel	
Item	

Section G2	**Intended Use and Indications for Use**

Information:	*Intended use refers to "the objective intent of the persons legally responsible for the labeling of devices. The intent is determined by such persons' expressions or may be shown by the circumstances surrounding the distribution of the article..." (see 21 CFR 801.4).*

Code of Federal Regulations

Enter the intended use of the product. *		
The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. ARK Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay. ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay. ARK Methotrexate Dilution Buffer is intended for the dilution of specimens for the ARK Methotrexate Assay.		
.	Intended Use	Prescription

Information:	*For AST devices, the Indication For Use statement should indicate: whether the assay is quantitative (MIC) or qualitative (breakpoint devices); whether results may be read and reported manually; which organism groups the device is indicated for testing; and any instrumentation the device may be used with, if applicable. A typical example of an intended use statement is: "ABC's system is intended for the in vitro qualitative or quantitative determination of antimicrobial susceptibility of rapidly growing aerobic non-fastidious Gram positive and Gram negative organisms on the ABC Instrument."*

Code of Federal Regulations

Information:	*Please attach the Indication(s) for Use Form to the following question.*

Indication(s) for Use Form

Enter the product's indications for use. *	
The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.	
File Attachment	Indications for use Form

Enter any special applications of the device or contraindications not addressed in the Intended Use Statement.
Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay

6.0 Manufacturing/Packaging/Sterilization Sites

Item: 1

Status Change *	No	
Site Operation		
.	Manufacturer *	Yes
.	Contract Manufacturer *	No
.	Contract Sterilizer *	No

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

.	Repackager/Relabeler	*	No

Contact Information	
Contact Information:	
Contact Name	Mr. Johnny Valdez
Occupation Title	President
Email Address	johnny@ark-tdm.com
Establishment Information:	
Establishment Name	ARK Diagnostics, Inc.
Division Name	
FDA Establishment Identifier (FEI)	
Central File Number (CFN)	
Registration Number	3005755244
Owner/Operator Number	10027663
D&B D-U-N-S Number	
Physical Location:	
Address	1190 Bordeaux Drive Sunnyvale, CA, 94089, US
Telephone Number	(408) 747-0700
Fax Number	(408) 747-0783
Mailing Location:	
Address	1190 Bordeaux Drive Sunnyvale, CA, 94089, US

7.0 Utilization of Standards

Information:	*Select the CDRH Recognized Standard from the available list.*

Select all standards referenced.

Item	Standard Title and Reference Number	Category	Organization
Item 1	Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)(EP 7-A)	InVitro	NCCLS
Item 2	Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision) (EP09-A2-IR)(EP09-A2-IR)	InVitro	NCCLS
Item 3	Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)(EP5-A)	InVitro	NCCLS

Standards Data Report for 510(k)s (FDA Form #3654, Form Approved OMB #0910-0120)

For each standard selected above, please fill out the Standards Data Report for 510(k)s (FDA Form #3654) and attach it here.

File Attachment 1	FDA-3654 EP5A2 Precision
File Attachment 2	FDA-3654 EP7A2 Specificity Interference
File Attachment 3	FDA-3654 EP9A2-IR Method Comparison
Details	

Did you reference any other standards?	Yes

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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7.1 Other Standards

Item: 1

Section I	**Utilization of Standards**

Standards Number
EP17A
Standards Organization
CLSI
Standards Title
Protocols for Determination of Limits of Detection and Limits of Quantitation
Version
Vol. 24 No. 34
Date: 10/01/2004

Please attach the standard. Before attaching, please verify any copyright restrictions.	
File Attachment	FDA-3654 EP17A Limit of Quantitation

Item: 2

Section I	**Utilization of Standards**

Standards Number
EP6A
Standards Organization
CLSI
Standards Title
Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
Version
Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
Date: 04/01/2003

Please attach the standard. Before attaching, please verify any copyright restrictions.	
File Attachment	FDA-3654_EP6A Linearity

8.0 Utilization of Guidance Documents

Please enter all referenced Guidance Documents.				
Item	Document Title	Office	Division	Web Page

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

9.0 Certification of Compliance with Clinical Trials

Information:	*The Food and Drug Administration has issued draft guidance titled: "Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of The Food and Drug Administration Amendments Act of 2007".*

The draft guidance can be found at: http://www.fda.gov/RegulatoryInformation/Guidances/ucm125335.htm

Form FDA-3674, ClinicalTrials.gov Data Bank

Information:	*Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) included a provision that all MA applications are required to be accompanied with certification that all applicable clinical trial information has been submitted to the ClinicalTrials.gov data bank.* *Beginning December 26, 2007, submissions must include form FDA-3674. If your submission includes data from a clinical trial, you must determine if your study is applicable for entry into the clinical trial registry data bank at ClinicalTrials.gov.*

Form FDA-3674, ClinicalTrials.gov Data Bank

For each clinical trial, complete form FDA-3674 and attach it here.

File Attachment	Clinical Trials Databank Form
Details	An in-house laboratory study was performed with leftover human specimens that are not individually identifiable. Clinical trials involving human subjects were not performed.

Section: OIVD Submission

OIVD 510(k)

OIVD 510(k) Submission

In vitro diagnostic (IVD) products are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body.

IVDs are medical devices as defined in section 210(h) of the Federal Food, Drug, and Cosmetic Act, and may also be biological products subject to section 351 of the Public Health Service Act. Like other medical devices, IVDs are subject to premarket and postmarket controls. IVDs are also subject to the Clinical Laboratory Improvement Amendments (CLIA '88) of 1988.

Is this an Antimicrobial Susceptibility Testing Device?	*	No

Is this a Collection Device?	*	No

Note:	*The 510(k) Short Form is intended for experienced submitters who are familiar with the Turbo 510(k) program and the level of detail that is required when completing the submission.*

Would you like to complete the OIVD 510(k) Short Form?	*	Yes

Section: 510(k) Short Form

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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1.0 Type of Product

Describe the type of product (Assay, Reagents, Instrument, or Calibrators and Controls), the device type (Quantitative, Qualitative, Numeric, or Non-numeric), and the device technology. *	
The product is comprised of reagents, calbrators, quality controls and dilution buffer. It is a quantitative, homogeneous enzyme immunoassay technology. Draft package inserts describing the product are attached.	
File Attachment 1	Package Insert for ARK MTX Calibrator
File Attachment 2	Package Insert for ARK MTX Control
File Attachment 3	Package Insert for ARK MTX Dilution Buffer
File Attachment 4	Package Insert ARK Methotrexate Assay

2.0 System Description

Describe the System Description. Include details about the Device Description, Modes of Operation, Software, Sample Identification, Specimen Sampling and Handling, Assay Types, Reaction Types, Calibration, Quality Control, and Principles of Operation. *	
ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay. Details for specimen handling, calibration and quality control are given in the appended package inserts (labeling) for the assay system.	
File Attachment	Package Insert ARK Methotrexate Assay

3.0 Substantial Equivalence

Note:	*"Click" the Display Hint button within the Question below for a sample table.*

Compare the similarities and differences between the device and the predicate device. Enter or attach a table that presents the information in the format of the example provided. *	
A comparison table is provided in the 510(k) Summary for the ARK Methotrexate Assay.	
File Attachment	510(k) Summary

4.0 Test Principle

Enter a brief description of the technology/methodology utilized in the device (i.e., describe how the device works). Describe the principles of the device methodology and whether it is well established or new for the intended use (provide references). Include relevant technical specifications of material and methods not included in the draft package insert (e.g., description of antibodies and epitopes). *	
The ARK Methotrexate Assay is a homogeneous enzyme immunoassay and uses a well established technology. The principle of the device is described in the package insert. Antibody specificity is described in Study G - Specificity.	
File Attachment 1	Study G Specificity
File Attachment 2	Package Insert ARK Methotrexate Assay

5.0 Performance Characteristics

Note:	*Provide the information described below as appropriate to support the substantial equivalence determination. The type of data to be provided in this section depends on the intended use, technological characteristics of the new device, and claims.*

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Note:	*If you followed CLSI guidelines, which have specific instructions and calculations for determining performance, you may certify this instead of describing all the specifics of your protocol, statistical analyses, and line data. You should clarify which specific aspects of the protocol you followed, describe your deviations from the protocol, and describe any manufacturer-specific choices not specified in the guideline (e.g. number of lots or sites for precision or partitioning of sub-populations for reference range evaluation). You should also provide a summary of the results as listed below. CLSI guidelines relevant for the following sections include: EP5, Evaluation of Precision Performance of Clinical Chemistry Devices; EP12, User Protocol for Evaluation of Qualitative Test Performance; EP6, Evaluation of the Linearity of Quantitative Analytical Methods; EP7, Interference Testing in Clinical Chemistry; EP9, Method Comparison and Bias Estimation Using Patient Samples; EP14, Evaluation of Matrix Effects; GP10, Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; C28, How to Define and Determine Reference Intervals in the Clinical Laboratory; M2, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard; M7 , Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard; M11, Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard; M23, Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline; M24, Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; Approved Standard; M27, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard and M100, Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement.*

5.1 Analytical Performance

Describe the Analytical Performance including Precision (Repeatability/Reproducibility), Linearity/Assay's Measuring (Reportable) Range, Calibrators and Controls (indicate if controls are included in the kit), Traceability, Stability, and Expected Values of Opened and Closed Controls and Calibrators; include the method by which the study was performed and the acceptance criteria; include Analytical Limits at Low Levels, Analytical Specificity, and Analytical Characterization of Cut-off. *	
Analytical performance of the ARK Methotrexate Assay is described in the 510(k) Summary, package insert, and study chapters appended here. Calibrators and Controls are provided separately. Dilution Buffer is provided separately. Calibration stability, on-board analyzer reagent stability, open calibrator/control stability and other product stability characteristics were studied. Cancer patients treated with methotrexate obtain high concentrations in serum/plasma. Performance of the dilution protocol and potential carryover from high concentration specimens to following low concentration specimens was studied. Matrix issues were studied. ARK deems the assay for methotrexate to be substantially equivalent to the predicate device., safe and effective for the intended use.	
File Attachment 1	Package Insert for ARK MTX Calibrator
File Attachment 2	Package Insert for ARK MTX Control
File Attachment 3	Package Insert for ARK MTX Dilution Buffer
File Attachment 4	Study A - Limit of Quantitation
File Attachment 5	Study B - Recovery
File Attachment 6	Study C - Linearity
File Attachment 7	Study D - Method Comparison
File Attachment 8	Study E - Precision
File Attachment 9	Study H - Specimen Stability
File Attachment 10	Study I - Carryover and Dilution Protocol
File Attachment 11	Study J - Calibrator Matrix
File Attachment 12	Study K - Anticoagulants
File Attachment 13	Study L1 - Calibration Curve Stability and On-Board Stability of Reagents
File Attachment 14	Study L2 - Open Container Stability for Calibrator and Control
File Attachment 15	Study M - Product Stability
File Attachment 16	Package Insert ARK Methotrexate Assay
File Attachment 17	Study F Interference
File Attachment 18	Study G Specificity
File Attachment 19	510(k) Summary

5.2 Comparison Studies Using Clinical Specimens

Note:	*For method comparison, describe bias/agreement between the new device and comparator (i.e., predicate, reference method, disease state or a combination there of as appropriate for the desired claim). This also includes a description of study design and statistical methods. For examples of information to be included in study design description, see the article reporting the STARD initiative (Clin Chem 2003; 49(1): 7-18).*

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Note:	*"Click" the Display Hint button within the Question below for sample tables.*

Describe the Method Comparison including the Study Design, Results, and Matrix Description and Comparison. *	
Method comparison studies are described in the appended Study D. Both serum and plasma specmens were tested in the analysis and method comparison was not dependent on serum/plasma matrix. Specimen matrix was evaluated in Study K - Anticoagulants and demonstrated equivalence of serum and plasma. Calibrator Matrix was evaluated in Study J and demonstrated equivalence to serum.	
File Attachment 1	Study D - Method Comparison
File Attachment 2	CAP Proficiency Sample Summary
File Attachment 3	Heath Control Proficiency Sample Summary
File Attachment 4	Study J - (b)(4) [redacted]
File Attachment 5	Study K - Anticoagulants

5.3 Clinical Studies (Where Applicable)

Note:	*Also see information in the "comparison studies using clinical specimens" section above as well as the CLSI GP10-A, Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots. Definition and rationale for "Clinical Truth," statistical methods applied.*

Enter the clinical sensitivity and clinical specificity: estimates of diagnostic accuracy with confidence intervals such as sensitivity, specificity, positive and negative predictive values, etc. Also include any other clinical supportive data, statistical analysis, and results, if applicable (e.g., other clinical supporting data such as clinical therapy monitoring data, etc.). *
NA

5.4 Clinical Cut-off

Describe the established cut-off and its validation for the new device. Define "equivocal zone" if applicable, and include a description of how results within this zone are reported to the user. *
NA

5.5 Reference Interval (Expected Values)

Enter the reference interval for this measurand and the method used to determine it. Include literature references if applicable. Describe the population studied (demographics, inclusion/exclusion criteria, number of individuals). Indicate if separate reference intervals for subclasses were clinically justified. Include the statistical method used to calculate ranges. *
NA

6.0 Labeling

Note:	*The minimum requirements below do not include requirements for instruments. Additional labeling recommendations may apply to Class II devices with special controls and Guidance.*

Provide proposed package labeling to demonstrate conformance to 21 CFR 809.10. Also, include package insert for the predicate device, if available. Labeling at a minimum should include: device components and specifications, reagent composition and important specifications, precautions/warnings, limitations, storage requirements, specimen handling and storage requirements, expiration/stability dating, instructions for reconstitution, mixing, and dilution, assay procedure, calibration, quality controls, results (calculations, formulas), results(interpretation), performance characteristics (summarize reproducibility, etc.), and study design (population studied, N, type of sample, matrix, dilution, target concentrations, etc.). *
Proposed package inserts are appended for ARK Methotrexate Assay, ARK Methotrexate Calibrator, ARK Methotrexate Control, ARK Methotrexate Dilution Buffer. Container label proofs are appended.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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File Attachment 1	Package Insert for ARK MTX Calibrator
File Attachment 2	Package Insert for ARK MTX Control
File Attachment 3	Package Insert for ARK MTX Dilution Buffer
File Attachment 4	Package Insert ARK Methotrexate Assay
File Attachment 5	Container Label Proofs

7.0 Other Supportive Information

Enter any additional supporting information for this submission such as: Risk analysis [Special 510(k)s], manufacturing information for critical reagents, copies of bibliography references, financial disclosures, and software validation or certification statements, if necessary, to support equivalence. *	
References used in package insert and in supporting documentation are appended.	
File Attachment 1	Abromowitch 1988
File Attachment 2	Al-Turkmani 2010
File Attachment 3	Albertioni 1996
File Attachment 4	Allen 1980
File Attachment 5	Anderson 1991
File Attachment 6	Belz 1994
File Attachment 7	Bleyer 1978
File Attachment 8	Blum 2002
File Attachment 9	Bore 1987
File Attachment 10	Breithaupt 1982
File Attachment 11	Buchen 2005
File Attachment 12	Chabner 1972
File Attachment 13	Chessels 1990
File Attachment 14	Collier 1982
File Attachment 15	Colom 2009
File Attachment 16	Dombrowsky 2011
File Attachment 17	Erttmann 1985
File Attachment 18	Flombaum 1999
File Attachment 19	Hann 1990
File Attachment 20	Hansen 1983
File Attachment 21	Hospira 2008
File Attachment 22	Jacobs 1976
File Attachment 23	Jacobs 1991
File Attachment 24	Jaffe 2008
File Attachment 25	Jonsson 1991
File Attachment 26	Leucovorin 2008
File Attachment 27	Martelli 2011
File Attachment 28	Mazanec 1989
File Attachment 29	Milano 1983
File Attachment 30	Powles 1991
File Attachment 31	Richmond 1997
File Attachment 32	Saeter 1991
File Attachment 33	Schwartz 2007

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

File Attachment 34	Stevens 1991
File Attachment 35	Triamterene
File Attachment 36	Wheeler 1991
File Attachment 37	Widemann 1995
File Attachment 38	Widemann 2006
File Attachment 39	Widemann 2010
File Attachment 40	Wolfrom 1990

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510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is _____.

807.92 (a)(1): Name: **ARK Diagnostics, Inc.**

Address: 1190 Bordeaux Drive
Sunnyvale, CA 94089

Owner Operator Number: 10027663
Establishment Registration: 3005755244

Phone: (408) 747-0700
FAX: (408) 747-0783
Contact: Kenneth C. Kasper, PhD – (408) 747-0708
Executive Director of Quality and Regulatory Affairs

Date prepared: June 30, 2011

807.92 (a)(2): Device name- trade name and common name, and classification

Trade name: ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control
ARK™ Methotrexate Dilution Buffer

Common Name: Homogeneous Enzyme Immunoassay

Classification: 21 CFR 862 Clinical Chemistry Test System - Toxicology;
Test Code LAO; Enzyme Immunoassay, Methotrexate
Pre-Amendment Device, Unclassified
(21 CFR 862.3200 DLJ, 21 CFR 862.3280 LAS)

807.92 (a)(3): Identification of the legally marketed predicate device

Abbott TDx®/TDxFLx® METHOTREXATE II (K932615)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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807.92 (a)(4): Device Description

The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and Methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

The ARK Methotrexate Assay consists of reagents R1 anti-Methotrexate polyclonal antibody with substrate and R2 Methotrexate labeled with bacterial G6PDH enzyme. The ARK Methotrexate Calibrator consists of a six-level set to calibrate the assay, and the ARK Methotrexate Control consists of a six-level set used for quality control of the assay (tri-level calibration range set and tri-level high range set). The ARK Methotrexate Dilution Buffer is equivalent to zero calibrator (Calibrator A).

807.92 (a)(5): Intended Use / Indications for Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

The ARK Methotrexate Calibrator is intended for the calibration of the ARK Methotrexate Assay.

The ARK Methotrexate Control is intended for the quality control of the ARK Methotrexate Assay.

The ARK Methotrexate Dilution Buffer is intended for the dilution of specimens containing high concentrations of methotrexate.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

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807.92 (a)(6): Technological Similarities and Differences to the Predicate

SUBSTANTIAL EQUIVALENCE COMPARATIVE CHART

Comparison between the ARK™ Methotrexate Assay and TDx®/TDxFLx® Methotrexate II Assay

Characteristic	**Device** ARK™ Methotrexate Assay	**Predicate** Abbott TDx®/TDxFLx® Methotrexate II (K932615)
Intended Use	The ARK™ Methotrexate Assay is intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers.	The TDx®/TDxFLx® Methotrexate II assay is a reagent system for the quantitative measurement of methotrexate, an antineoplastic drug, in serum or plasma.
Indications for Use	The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.	The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.
Sample	Serum or plasma	Serum or plasma
Methodology	Homogenous enzyme immunoassay (EIA)	Fluorescence polarization immunoassay (FPIA)
Reagent Components	Two (2) reagent system: Anti-Methotrexate Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to Methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers Enzyme Reagent (R2) containing Methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	Reagent Pack: W = Wash solution, solvent, sodium azide S = Methotrexate Antibody (mouse monoclonal), buffer, protein and sodium azide T = Methotrexate Fluorescein Tracer, buffer, protein, surfactant and sodium azide P = Pretreatment Solution, protein, surfactant and sodium azide
Platform required	Automated clinical chemistry analyzer	TDx clinical chemistry analyzer
Accessory reagents	Calibrators (six levels) and controls (six levels) in a synthetic matrix; Dilution Buffer	Calibrators (six levels) and controls (six levels) in human serum; Dilution Buffer
Testing environment	Routine clinical laboratory	Routine clinical laboratory
Reagent condition and storage	Liquid, 2-8˚ C	Liquid, 2-8˚ C

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807.92 (b)(1) and 807.92 (b)(2):
Brief Description of Nonclinical and Clinical Data

Limit of Quantitation (LOQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	**MTX Concentration (µmol/L)**
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

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Accuracy

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Target concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

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Assay Range

The range of the ARK Methotrexate Assay calibration curve is 0.00 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen into the range of the calibration curve. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the calibration range.

Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarized Immunoassay method (monoclonal FPIA).

Methotrexate concentrations by FPIA ranged 0.02 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.00 to 1500 µmol/L. Results of the Passing-Bablok regression analysis for the study are shown below (with 95% confidence limits) for 111 specimens within the calibration range as well as for all 157 specimens in the extended range.

Parameter	**Range 0.02 to 0.97 µM**		**Range 0.02 to 1440 µM**	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.01 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.971	(0.958 to 0.980)	0.998	(0.997 to 0.998)
Number of Samples	111	na	157	na

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within-run, between-day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.10 µmol/L, SD ≤0.01 at ≤0.10 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		**Methotrexate (~ 0.50 µmol/L)**	
Interfering Substance	**Interferent Concentration**	**Serum Control**	**Test**	**Serum Control**	**Test (% Control)**
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity - Drug Interference

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-Hydroxymethotrexate.

Crossreactivity to the minor, inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA)

The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for methotrexate cancer treatement due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

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Compound	Tested (µmol/L)	Methotrexate Absent		Methotrexate Present 0.05 µmol/L		Methotrexate Present 0.50 µmol/L	
		MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

Anticoagulants

Studies were conducted to determine the performance characteristics of the assay for both serum and plasma samples containing methotrexate.

The results indicate that there is no significant difference between the recovery of methotrexate in serum or plasma.

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Sample Stability

Human specimens were shown to be stable frozen (at least 15 months), forty-eight (48) hours at room temperature, refrigerated (at least 21 days) and after three (3) successive freeze/thaw cycles.

On-Board Stability

Calibration Curve Stability: A stored calibration was effective up to at least 19 days based on supporting data.

Reagent on-board stability: Reagents were effective when stored after transfer to analyzer specific reagent containers for up to at least 25 days based on supporting data. In-use stability of calibrator and controls was also demonstrated.

807.92 (b)(3): Conclusions from Nonclinical Testing

As summarized above, the ARK Methotrexate Assay System, including the ARK Methotrexate Calibrator, ARK Methotrexate Control and ARK Methotrexate Dilution Buffer, is substantially equivalent to the Abbott TDx®/TDxFLx® METHOTREXATE II Assay system. The ARK Methotrexate Assay system was shown to be safe and effective for its intended use based on performance studies.

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Medical and Pediatric Oncology 16:297–303 (1988)

High-Dose Methotrexate Improves Clinical Outcome in Children With Acute Lymphoblastic Leukemia: St. Jude Total Therapy Study X

Minnie Abromowitch, MD, Judith Ochs, MD, Ching-Hon Pui, MD, David Kalwinsky, MD, Gaston K. Rivera, MD, Diane Fairclough, PhD, A. Thomas Look, MD, H. Omar Hustu, MD, Sharon B. Murphy, MD, William E. Evans, PharmD, Gary V. Dahl, MD, and W. Paul Bowman, MD

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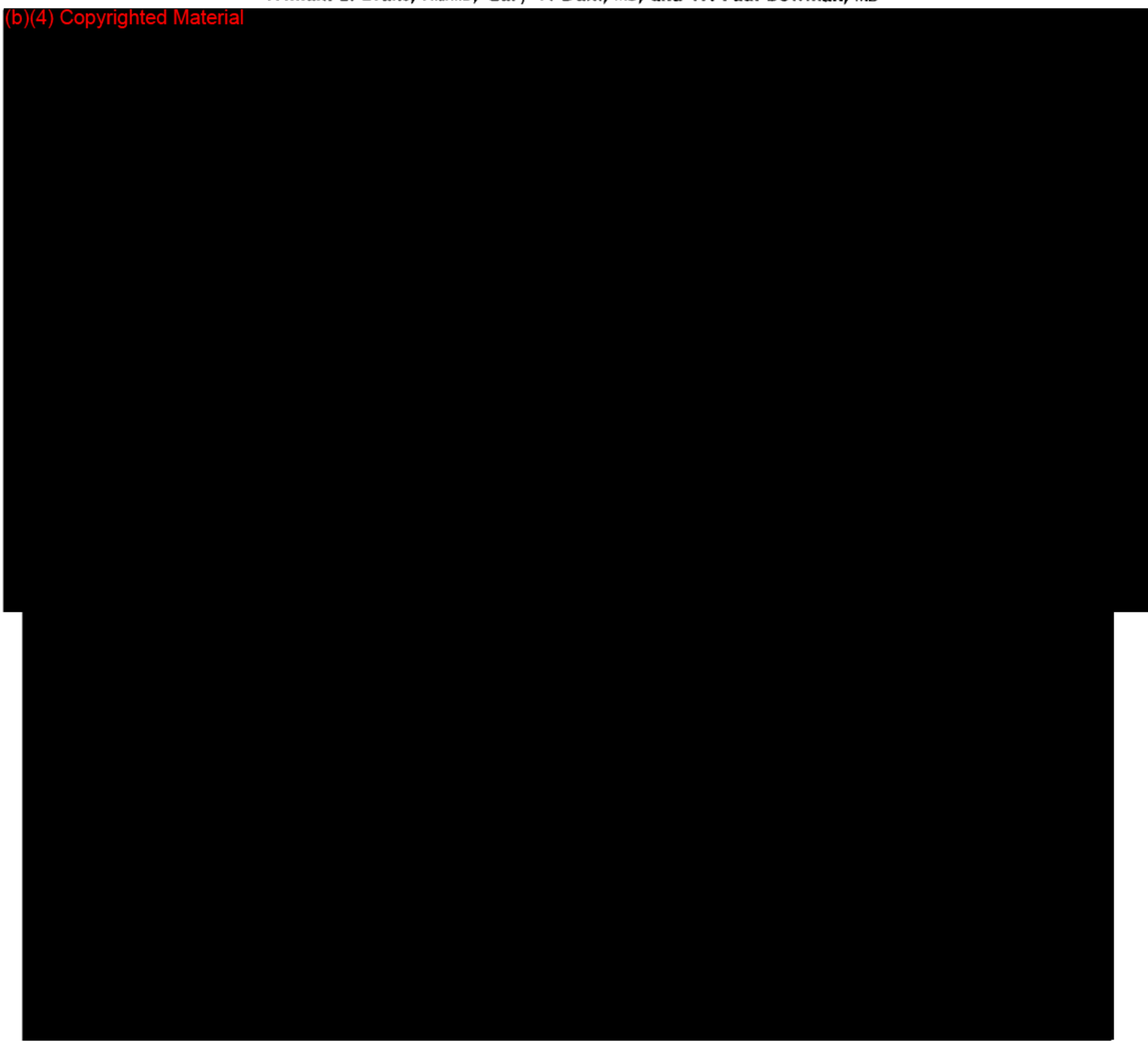

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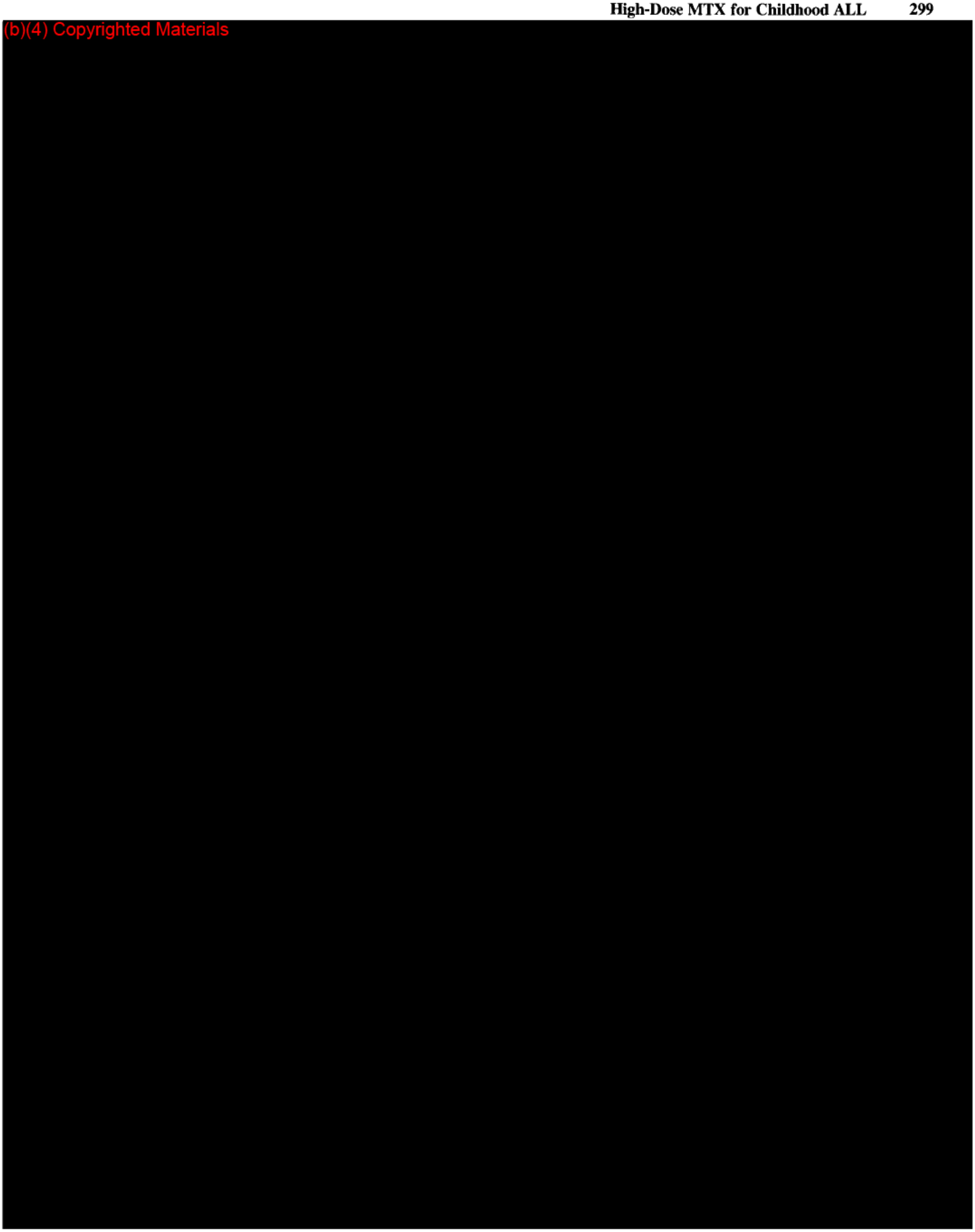

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Clinical Chemistry 56:12
1792–1796 (2010)

Difficulty Measuring Methotrexate in a Patient with High-Dose Methotrexate–Induced Nephrotoxicity

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CASE

An 18-year-old male presented with pain and swelling in his left leg that he noted after playing football. An x-ray of the affected leg showed a destructive lesion that prompted a concern for malignancy. Subsequent tests, including magnetic resonance imaging, a bone scan, and a needle biopsy of the lesion, confirmed nonmetastatic osteosarcoma in the left proximal tibia. The patient was started on a standard regimen of chemotherapy. He received 4 cycles of high-dose methotrexate (HDMTX)[3] with leucovorin rescue and 2 cycles of cisplatin and doxorubicin, which he tolerated well. Each HDMTX course involved the intravenous administration of 20 g methotrexate (MTX) over 4 h. The patient experienced delayed MTX clearance after the first cycle but showed typical clearance after the subsequent 3 cycles. He then underwent a planned radical resection of the tumor with allograft placement. After the patient recovered from surgery, chemotherapy resumed, and the patient received 2 additional cycles of cisplatin and doxorubicin and 1 additional cycle of HDMTX. His treatment was interrupted when he had to undergo surgery for a wound infection in the affected leg. After recovery from the second surgery, the patient received a sixth HDMTX cycle. After this cycle, the patient developed acute nephrotoxicity, which was manifested by marked renal dysfunction and delayed MTX clearance. His plasma creatinine concentration increased from 0.8 mg/dL (8 mg/L) at the start of the cycle to 6.8 mg/dL (68 mg/L) after he received HDMTX. Plasma MTX concentrations were 1700 μmol/L at 24 h after infusion, 450 μmol/L at 48 h, and 350 μmol/L at 72 h. The patient was treated with aggressive hydration, diuresis, and 1500 mg leucovorin intravenously every 6 h for several days. These measures did not reduce MTX below the toxic concentration, however, and the decision was made to give the patient glucarpidase [carboxypeptidase G_2 ($CPDG_2$); BTG International] 4 days after he received the HDMTX infusion.

QUESTIONS TO CONSIDER

1. What is the incidence of MTX-induced nephrotoxicity, and how is it treated?
2. What is the mechanism of $CPDG_2$ action, and what is its clinical utility?
3. How is MTX measured, and what is the source of the discrepancy in the patient's MTX measurements?

The laboratory experienced difficulty in reporting subsequent plasma MTX concentrations because of discrepancies in the Abbott TDx immunoassays of the MTX concentrations in serially diluted samples. For example, the plasma MTX concentration for a sample obtained after $CPDG_2$ administration and diluted with 9 volumes of diluent (10-fold dilution) was 9.6 μmol/L, whereas the measured concentration was 50 μmol/L for the same sample diluted with 99 volumes of diluent (100-fold dilution).

Because MTX could not be measured accurately and because of concern for ongoing MTX toxicity, a second $CPDG_2$ dose was administered to the patient 2 days after the first. Five days later, the discrepancy in MTX measurements disappeared, and the laboratory was able to report subsequent plasma MTX concentrations, which were <4.5 μmol/L. Because MTX and creatinine concentrations were decreasing steadily, the decision was made to complete intravenous hydration at 170 mL/h and leucovorin rescue with 250 mg administered intravenously every 6 h at home until the MTX concentration was <0.1 μmol/L.

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Received February 4, 2010; accepted June 15, 2010.
Previously published online at DOI: 10.1373/clinchem.2010.144824
[3] Nonstandard abbreviations: HDMTX, high-dose methotrexate; MTX, methotrexate; $CPDG_2$, carboxypeptidase G_2; DAMPA, 2,4-diamino-N^{10}-methylpteroic acid; FPIA, fluorescence polarization immunoassay; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

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DISCUSSION

HDMTX, defined as MTX doses ≥1000 mg/m^2 administered by prolonged intravenous infusion followed by leucovorin rescue, has been widely used in the treatment of malignancies such as osteosarcoma, acute lymphoblastic leukemia, and lymphoma. MTX is metabolized primarily to 7-hydroxymethotrexate, the plasma concentrations of which exceed those of the parent compound shortly after HDMTX infusion *(1)*. MTX-induced acute nephrotoxicity is thought to be due to the precipitation of MTX or its insoluble metabolites in the renal tubules or to a direct toxic effect of MTX on the tubules *(2)*. Renal dysfunction causes delayed MTX elimination and unsuccessful rescue by leucovorin. Nephrotoxicity has been reported in clinical trials in approximately 1.8% of patients with osteosarcoma treated with HDMTX. The mortality rate is 4.4% among these patients *(3)*. MTX concentrations >10 μmol/L at 24 h, >1 μmol/L at 48 h, or >0.1 μmol/L at 72 h after infusion are associated with a high risk for nephrotoxicity *(2)*.

HDMTX-induced nephrotoxicity is conventionally managed through hydration and alkalinization of the urine to enhance the solubility and urinary excretion of MTX. Other measures include monitoring of blood creatinine and MTX concentrations, as well as pharmacokinetically guided leucovorin rescue to restore intracellular folate concentrations. Dialysis has also been used for MTX removal.

$CPDG_2$ is a bacterial enzyme available in a recombinant form cloned from *Pseudomonas* strain RS-16. $CPDG_2$ rapidly hydrolyzes the C-terminal glutamate from MTX to form the inactive compounds 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) and glutamate, thus providing a rapid route of elimination. $CPDG_2$ administration in combination with thymidine and leucovorin is highly effective, decreasing plasma MTX concentration by 95%–99% within 15 min in patients with HDMTX-induced nephrotoxicity *(4, 5)*.

In a patient with an increased plasma MTX concentration and poor renal function, it is crucial to use a rapid means of eliminating MTX from the circulation to prevent further renal damage and to avoid other toxicities, such as myelosuppression and mucositis, that are associated with increased MTX concentrations. $CPDG_2$ administration is recommended for patients whose plasma MTX concentration is >10 μmol/L by 42–48 h after beginning MTX infusion *(2)*. The patient in this case clearly met the criteria for receiving $CPDG_2$.

Plasma MTX concentrations are routinely monitored after HDMTX treatment to determine the rate of drug clearance and the leucovorin dose required for rescue. The method most commonly used for routine MTX measurement in clinical laboratories is fluorescence polarization immunoassay (FPIA). In addition to immunoassays, capillary zone electrophoresis and HPLC methods have been described for measuring MTX and its metabolites in biological fluids *(4)*. A dihydrofolate reductase enzyme-inhibition assay that uses a 96-well plate reader to measure the plasma MTX concentration has also been introduced *(5)*.

Because of the limited analytical range of the MTX assay used in our laboratory and the wide range of concentrations observed in our patients, our standard operating procedure involves the serial dilution (10-fold, 100-fold, 500-fold) of samples with saline. The discrepancy in this patient's serially diluted MTX measurements was due to differing concentrations of the interfering MTX metabolite DAMPA, which was present at a high concentration after $CPDG_2$ treatment. The package insert for the MTX assay states that when MTX and DAMPA are both present, the interference from DAMPA is less (an approximately 26% false increase) than when only DAMPA is present (up to a 59% false increase). Thus, the results for the 10-fold dilutions in this case back-calculated to lower MTX concentrations than those for the 100-fold dilutions because the DAMPA interference was reduced by the presence of MTX. The amount of MTX present in the 100-fold dilutions was well below the linear interval of the assay and allowed greater interaction between the antibody and DAMPA, thereby increasing the observed MTX concentration. This variable interference further limits the utility of FPIA after $CPDG_2$ administration.

DAMPA is typically a minor metabolite of MTX, and its plasma concentration after HDMTX infusion is usually very low *(6)*. The DAMPA interference of MTX immunoassays is well established, however. The MTX FPIA has shown 82.6% cross-reactivity with DAMPA in the method that uses polyclonal antibodies and 41.1% cross-reactivity in the method that uses monoclonal antibodies *(7)*. DAMPA interference causes marked MTX overestimation and makes immunoassays unreliable for MTX measurement after $CPDG_2$ treatment. Unlike DAMPA, the cross-reactivity of 7-hydroxymethotrexate with the MTX FPIA is only 0.6% *(8)*. In the MTX FPIA, one would expect similar low cross-reactivity with the hydroxylated metabolite of DAMPA and the glucuronide of that molecule; however, the degree of interference may be different in polyclonal antibody–based or other forms of the immunoassay.

After the clinical laboratory staff suspected interference in the MTX assay, they contacted the

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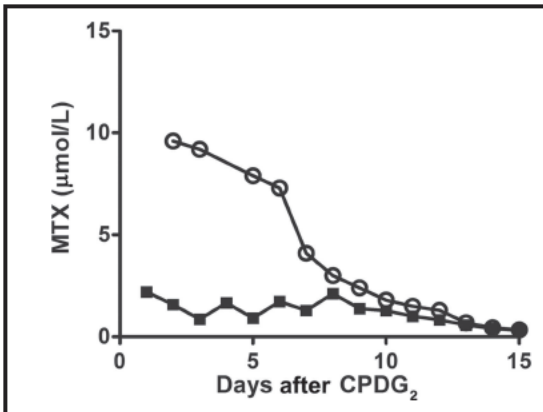

Fig. 1. Plasma MTX concentration measured by FPIA (10-fold dilution, Abbott TDx assay) (○) and LC-MS/MS (■) after $CPDG_2$ administration.

medical team and learned that the patient had received $CPDG_2$. To confirm the DAMPA interference and to have the ability to accurately measure MTX concentrations after $CPDG_2$ administration, we subsequently developed a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method for MTX measurement *(9)*. Use of this LC-MS/MS method to measure MTX concentrations in samples from the presented patient confirmed the DAMPA interference in the FPIA: Plasma MTX concentrations obtained by LC-MS/MS were markedly lower than those obtained with the TDx analyzer with a 10-fold dilution (Fig. 1). LC-MS/MS measurements of MTX also indicated that the first $CPDG_2$ dose was effective in reducing the plasma MTX concentration by 99% (from 230 μmol/L to 2.2 μmol/L). After DAMPA clearance from the patient's circulation by 7 days after $CPDG_2$ administration, MTX concentrations measured by FPIA were comparable to those obtained by LC-MS/MS (Fig. 1).

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

POINTS TO REMEMBER

- Nephrotoxicity has been reported in approximately 1.8% of patients with osteosarcoma treated with HDMTX in clinical trials, with a mortality rate of 4.4% among these patients.
- HDMTX-induced nephrotoxicity is conventionally managed through hydration and alkalinization of the urine to enhance the solubility and urinary excretion of MTX. Dialysis has also been used for MTX removal.
- $CPDG_2$ rapidly hydrolyzes the C-terminal glutamate of MTX to form the inactive metabolite DAMPA and glutamate, thus providing a rapid route of elimination.
- The method most commonly used for routinely measuring MTX in clinical laboratories is FPIA. The DAMPA metabolite exhibits high cross-reactivity with most MTX immunoassays.
- The laboratory should educate physicians about the interference caused by $CPDG_2$ and the inability of immunoassays to accurately measure MTX in $CPDG_2$-treated patients.
- It is critical that the clinical team notify the laboratory when $CPDG_2$ is administered to avoid the reporting of falsely increased MTX concentrations that would indicate failure of $CPDG_2$ therapy.

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Clinical Chemistry 42:1
39–44 (1996)

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Evaluation of clinical assays for measuring high-dose methotrexate in plasma

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Four routine assays commonly used for monitoring plasma methotrexate (MTX) during high-dose therapy were validated by HPLC as the comparison method. MTX and its main metabolite, 7-hydroxymethotrexate (7-OHMTX), were analyzed by HPLC with postcolumn derivatization and fluorometric detection. About 200 clinical plasma samples from 13 children with acute lymphoblastic leukemia who received 5–8 g/m^2 MTX as 24-h infusions were analyzed. The fraction of measured concentrations of MTX that were within 75–125% of the values obtained by HPLC were 64.5% for enzyme inhibition assay, 56.4% for fluorescence polarization immunoassay with polyclonal antibodies (FPIA1; Abbott), 58.9% for FPIA2 (with monoclonal antibodies; Abbott), and 46.4% for enzyme-multiplied immunoassay (Emit™; Syva). All nonchromatographic procedures were subject to interferences from MTX plasma metabolites or endogenous substances. The interference from 7-OHMTX was, however, somewhat less pronounced for FPIA2 (monoclonal) than for FPIA1 (polyclonal).

INDEXING TERMS: chemotherapy • intermethod comparison • enzyme inhibition assay • enzyme immunoassay • fluorescence polarization immunoassay • chromatography, liquid

Intravenous high-dose methotrexate (MTX; 2,4-diamino-N^{10}-methylfolic acid) therapy followed by leucovorin (folinic acid) rescue is widely used in the chemotherapy of various neoplastic diseases, such as acute leukemia and osteogenic sarcoma *[1]*.[5] Monitoring of plasma MTX concentrations is mandatory in such protocols to identify patients with delayed elimination to individualize leucovorin doses to reduce toxicity.

Several assays suitable for clinical use are available for quantifying MTX in plasma. HPLC with ultraviolet or fluorescence detection *[2–7]* is widely used in research laboratories and offers sensitivity, accuracy, selectivity, and the ability to quantify MTX as well as its metabolites in biological fluids. However, chromatographic methods are more time consuming than such automated methods as fluorescence polarization immunoassay (FPIA), radioimmunoassay (RIA), enzyme inhibition assay (EIA), and enzyme-multiplied immunoassay (Emit™; Behring Diagnostics, Syva Business, San Jose, CA)—methods all currently used for routine monitoring of MTX. These methods require limited sample pretreatment and allow laboratories to produce assay results in a short time. However, discrepancies between immunoassays and HPLC have been reported in many studies *[8–13]*, most probably resulting from interference of metabolites and possibly endogenous substances.

MTX is mainly metabolized extracellularly to 7-hydroxymethotrexate (7-OHMTX) and to some extent 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) *[9, 14, 15]*. Although these metabolites have much lower affinity for dihydrofolate reductase, they could influence the determination of MTX because of the close structural similarities (Fig. 1). Recently, a new kit (FPIA2) has become available from Abbott Labs. (Abbott Park, IL) for measuring MTX in plasma; in an attempt to improve assay specificity, this kit uses a monoclonal antibody instead of the polyclonal antibodies of the current assay (FPIA1).

The aim of this study was to evaluate four different clinical MTX assays (Emit, FPIA1, FPIA2, and EIA) in comparison with HPLC for analyses of patients' samples.

Materials and Methods

CHEMICALS

MTX [(+)-amethopterin] was obtained from Sigma Chemical Co. (St. Louis, MO), and 7-OHMTX was generously donated by W. E. Evans, St. Jude Children's Research Hospital, Memphis, TN. DAMPA was prepared enzymatically by treating (+)-amethopterin with carboxypeptidase G *[16]*. After purification by preparative HPLC, we determined the concentration of

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[5] Nonstandard abbreviations: MTX, methotrexate; 7-OHMTX, 7-hydroxymethotrexate; EIA, enzyme inhibition assay; FPIA, fluorescence polarization immunoassay; DAMPA, 2,4-diamino-N^{10}-methylpteroic acid; ALL, acute lymphoblastic leukemia; and NOPHO, Nordic Association for Paediatric Hematology and Oncology.

Received March 15, 1995; accepted October 6, 1995.

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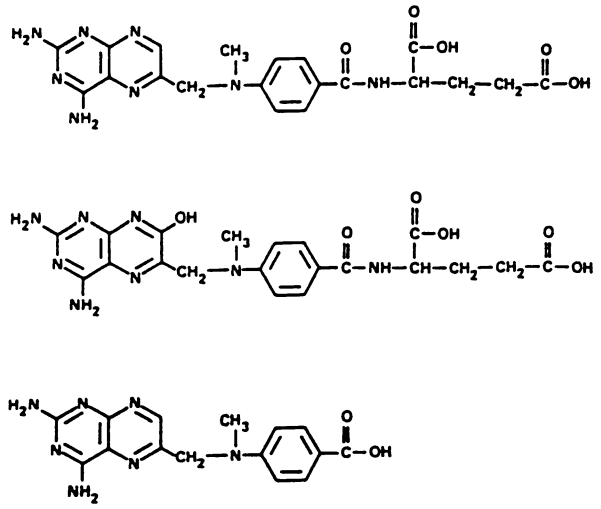

Fig. 1. Structures of MTX (*upper*) and its plasma metabolites, 7-OHMTX (*middle*) and DAMPA (*lower*).

DAMPA *[14]*. Other chemicals used in this study were of analytical purity and obtained commercially.

CLINICAL SAMPLES

Plasma from 42 MTX infusions in 13 patients (ages 3.3–12.9 years) with acute lymphoblastic leukemia (ALL) was analyzed. Each patient was treated 1–5 times with 5–8 g/m^2 doses of MTX. Ten percent of the dose was administered during 1 h and the rest during constant-rate infusion for 23 h [according to the 1992 protocol of the Nordic Association for Paediatric Hematology and Oncology (NOPHO)]. Leucovorin rescue (15 mg/m^2) was given intravenously at 36, 42, 48, and 54 h after the start of the infusion. In cases of delayed MTX elimination, the leucovorin doses are increased. All patients were hydrated with 3000 mL/m^2 of 50 g/L glucose solution containing 40 mmol/L sodium bicarbonate and 20 mmol/L potassium chloride.

Blood samples were collected at 20 (steady-state concentration), 36, 42, 54, and 66 h after the start of the infusion. All blood samples were drawn from a central venous catheter except for the steady-state sample, which was taken from a peripheral vein. Blood samples were collected in heparin-coated glass containers and kept refrigerated, protected from light, for up to 12 h until centrifugation (900*g*, 15 min, 4 °C). Plasma samples were stored at −80 °C in polypropene tubes until analysis.

PROCEDURES

HPLC. The HPLC was performed at 22 °C with a CM4000 pump (Milton Roy, LDC Div., Riviera Beach, FL), a CMA-240 autosampler (Carnegie Medicine, Stockholm, Sweden), a beam-boost photochemical reactor (ICT HG, Frankfurt, Germany) equipped with a 254-nm lamp and a 10 m × 0.3 mm reaction coil and used at an operating temperature of 37 °C, and a Shimadzu 551 fluorescence detector (Shimadzu Corp., Kyoto, Japan) with excitation and emission wavelengths of 350 and 435 nm, respectively. For the solid-phase extraction, a VacMaster (International Sorbent Technology, Mid-Glamorgan, UK) was used. MTX and 7-OHMTX were extracted from plasma by a previously published HPLC method *[5]*, which we slightly modified. Solid-phase extraction cartridges (Isolute HAX 200 mg, part No. 903-0020-B; International Sorbent Technology) were conditioned before use with 3 mL of methanol and 3 mL of 0.1 mol/L phosphoric acid. We diluted 0.01–1 mL of plasma with 2 mL of 0.1 mol/L phosphoric acid and applied this to the cartridge with a flow rate of ~2.5 mL/min. We then washed the cartridge with 2 mL of 50 mL/L methanol in 0.1 mol/L phosphoric acid and 3 mL of Na_2HPO_4 (pH 8.6), washed it with 50 mL/L methanol in water, and dried it by aspirating air for 2 min. The cartridge was finally eluted with 2 mL of 20 g/L trifluoroacetic acid in methanol. The eluate was collected in glass test tubes and evaporated to dryness under a stream of nitrogen in a water bath (40 °C). The residue was finally dissolved in 100 μL of 0.1 mol/L phosphoric acid, and 20 μL of this was injected into the chromatograph.

Standard samples, prepared by adding to blank plasma known amounts of MTX and 7-OHMTX, were used to construct calibration curves. The calibration curves were linear over the concentration range of 0.001–0.5 μmol/L for MTX and 7-OHMTX. Samples with higher plasma concentrations were diluted with 0.1 mol/L phosphoric acid before injection. A C_{18} column (80 × 4.6 mm, 3 μm; Perkin-Elmer, Norwalk, CT) was used. A mobile phase consisting of 0.1 mol/L phosphate buffer, pH 6.5, with (per liter) 65 mL of acetonitrile and 2 mL of 30% hydrogen peroxide was delivered at a flow rate of 1 mL/min. Peak area measurements and baseline integrations were performed on a Macintosh Classic computer (Apple, Chicago, IL) equipped with Chromac 3.1. software (Drew, London, UK).

The within-day repeatability and day-to-day reproducibility of the method were assessed by analysis of the pooled blank plasma supplemented with known amounts of MTX and 7-OHMTX at different concentrations, as shown in Table 1. The limit of quantification was determined for 1-mL plasma samples as the concentration giving a signal-to-noise ratio (S:N) of 10:1, with CV <10% (Table 1). The within-day and day-to-day precision of the HPLC method in the measurement of 7-OHMTX at the concentrations indicated in Table 1 were <9.4% (data not shown).

Table 1. Sensitivity, precision, and cross-reactivity of methods.

	MTX, μmol/L		CV, % (n)		
Method	Limit of quantification	Added	Within-run	Between-run	Cross-reactivity with DAMPA,[a] % (n)
HPLC	0.0001[b]	0.001	3.0 (4)	—	—
		0.010	5.2 (13)	7.7 (8)	—
		0.100	6.2 (13)	6.1 (8)	—
		1.500	9.9 (10)	10.5 (10)	—
EIA	0.010[b]	1.500	1.7 (10)	3.2 (6)	1.8 (4)
Emit	0.300[c]	1.500	3.0 (10)	2.9 (9)	99.8 (5)
FPIA1	0.050[c]	1.500	1.4 (10)	7.1 (11)	82.6 (4)
FPIA2	0.050[c]	1.500	1.1 (10)	4.5 (11)	41.1 (4)

[a] 100 × ([DAMPA]$_{Method}$ − [DAMPA]$_{HPLC}$)/[DAMPA]$_{HPLC}$.
[b] Used limit.
[c] Recommended by the manufacturers.

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FPIA. Measurement of MTX in plasma by FPIA, with polyclonal (FPIA1) and monoclonal antibodies (FPIA2), was performed on an FLx TDx analyzer (Abbott Scandinavia AB, Diagnostics Division, Stockholm, Sweden) according to the assay manual. The FPIA kits for analysis of MTX were kindly donated by Abbott Scandinavia.

Emit. Emit assays were performed on an automated drug analyzer (Behring Diagnostics, Syva Business, San Jose, CA). The Emit kits were kindly donated by Syva Scandinavia, Syntex Diagnostics, Skärholmen, Sweden.

EIA. EIA was done according to Scheufler *[17]*, as modified by Schroeder and Heinsvig *[18]*.

METHOD COMPARISONS

Plasma samples were received in the laboratory for the measurement of MTX and were analyzed directly by EIA as a part of a clinical routine. All samples were subsequently analyzed with methods outlined above within 8 months. Plasma samples were analyzed with FPIA1, FPIA2, and Emit with the same batches of reagents and dilution protocols during a period of 2 days. MTX concentrations <1 μmol/L were analyzed directly without sample dilution. Higher concentrations of plasma MTX were analyzed after dilution.

The within-day repeatability and day-to-day reproducibility of the methods in the measurement of MTX were examined in pooled plasma samples with 1.5 μmol/L added MTX on day 1 and on consecutive days in all clinical methods used (Table 1). To determine the cross-reactivity of DAMPA, we added 1.5 μmol/L DAMPA and 1.5 μmol/L MTX to plasma samples before analysis (Table 1).

STATISTICAL EVALUATION

Method-comparison data were evaluated by the graphic principle, described in detail previously *[19]*. Briefly, the ratio of concentration data obtained by analysis from the two procedures being compared was plotted vs the data from the comparison procedure or vs the concentration of compounds suspected to cause interferences with one of the analytical procedures. Median values of the ratios and the 25th and 75th percentiles were calculated as given in Ott and Mendenhall *[20]*. Correlations were established by the Kendall rank correlation test *[21]*. The mean cross-reactivity values of DAMPA in the methods were compared by paired *t*-test. A probability value ≤0.05 was considered statistically significant.

Results

Limits of quantification. The recommended lower limit of quantification for the compared analytical procedures are presented in Table 1. HPLC is the most sensitive assay for measuring MTX.

Precision. Within- and between-run precisions of the methods for drug-supplemented plasma samples are presented in Table 1. The CVs were <11% for all assays, with HPLC having the greatest.

Method comparison. The results from MTX analyses by various analytical procedures as compared with HPLC results are illustrated in Fig. 2(A-D). At low MTX concentrations (i.e., a long time after drug infusion), Emit, FPIA1, and, to a lesser extent, FPIA2 tend to overestimate the MTX concentration.

The statistical analyses performed to assess the degree of accuracy of the methods are listed in Table 2. The fraction of measured concentrations of MTX within 75–125% of the values

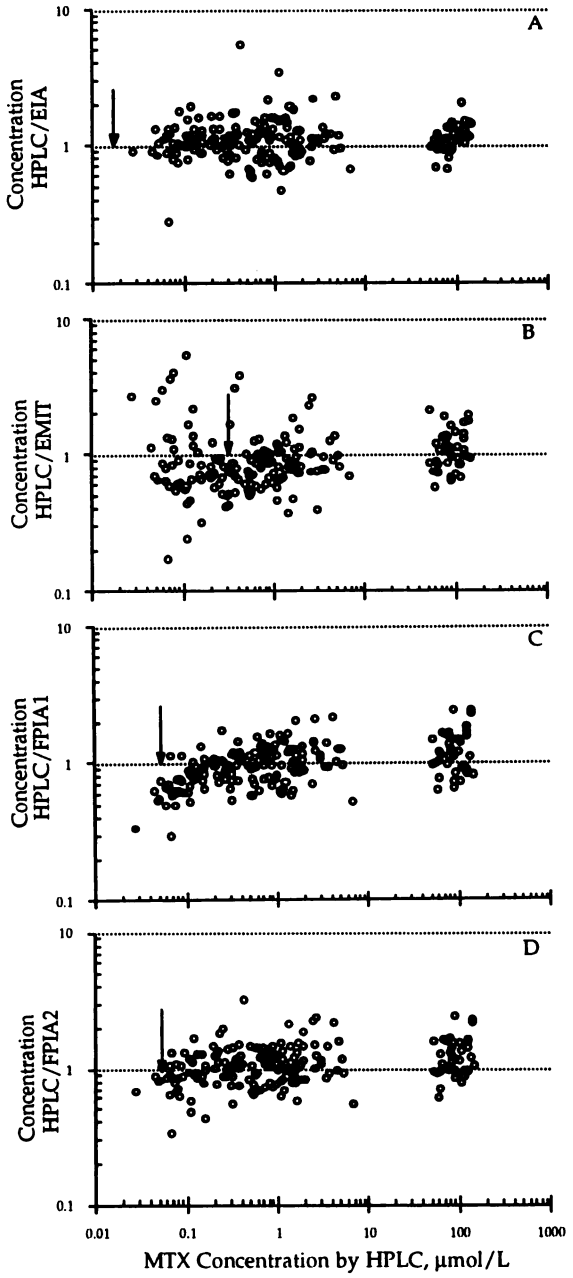

Fig. 2. Evaluation of nonchromatographic procedures for quantification of MTX in plasma: relation between ratios of HPLC with EIA (*A*), Emit (*B*), FPIA1 (*C*), and FPIA2 (*D*) in the measurement of MTX plasma concentration with HPLC.

Arrows show the limits of quantification.

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Table 2. Quantitative profile of methods comparisons.

	Compared analytical techniques				
	HPLC/EIA	HPLC/Emit	HPLC/FPIA1	HPLC/FPIA2	FPIA1/FPIA2
Median[a]	1.098	0.852	0.920	1.067	1.142
25th percentile[a]	0.919	0.671	0.962	0.875	0.976
75th percentile[a]	1.300	1.078	1.191	1.298	1.218
Fraction within 0.75–1.25[b]	127/197 (64.5)	90/194 (46.9)	111/197 (56.4)	116/197 (58.9)	145/197 (73.6)

[a] Ratio of MTX concentrations determined by the methods compared.
[b] No./total no. (and %).

obtained by HPLC was 64.5% for EIA, 56.4% for FPIA1, 58.9% for FPIA2, and 46.9% for Emit (Table 2).

Cross-reactivity. A significant influence ($P < 0.05$) of 7-OHMTX on MTX measurement was seen with all nonchromatographic assays (Fig. 3, A-D). The influence of DAMPA in the MTX assays is listed in Table 1. In the Emit and EIA assays, DAMPA cross-reacted by 99.8% and 1.8%, respectively.

Comparison of FPIA1 with FPIA2. The relation between MTX concentrations obtained by FPIA1 and FPIA2 is demonstrated in Fig. 4A. The cross-reactivity of FPIA2 with 7-OHMTX was less pronounced than of FPIA1 (Fig. 4B). FPIA2 showed lower cross-reactivity of DAMPA with MTX than did FPIA1 (41.1% vs 82.6%; $P < 0.001$).

Discussion

MTX is the only anticancer drug for which plasma concentrations are routinely monitored in patients to guide the administration of an antidote (leucovorin). It has been suggested that the therapeutic effect of MTX is based on a balance of exposure to MTX and leucovorin rescue *[22]*. According to the 1992 protocol of NOPHO, ALL patients with plasma MTX concentrations >1 μmol/L at 42 h or >0.2 μmol/L at 66 h after the start of infusion are considered to be at high risk of developing toxicity and are therefore given intensified leucovorin treatment. Because these concentrations are used as cutoff values, it is important to quantify them accurately. In this study the leucovorin doses given were not in accordance with the actual concentration measured by HPLC in 12, 6, 4, and 8 courses as measured with Emit, FPIA1, FPIA2, and EIA, respectively (Table 3). The difference was greater than could be explained by the inter- and intraassay variabilities of the HPLC method (CV 11%) used in the present study as the comparison method.

Both FPIA and Emit immunoassays overestimated the plasma concentrations of MTX in the terminal phase of the plasma concentration–time curves to a certain extent (Figs. 2 and 3). Emit seems to have a more extensive cross-reactivity of antibodies with metabolites relative to FPIA. The recommended

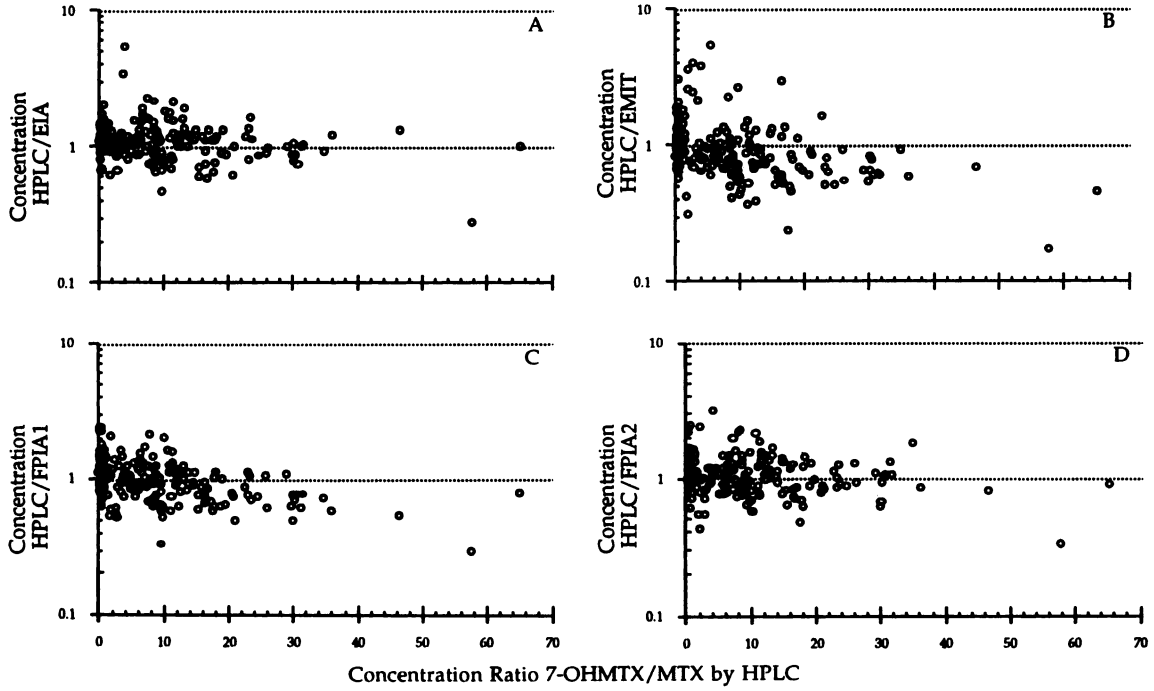

Fig. 3. Influence of the metabolite, 7-OHMTX, on MTX analysis by EIA (*A*), Emit (*B*), FPIA1 (*C*), and FPIA2 (*D*).

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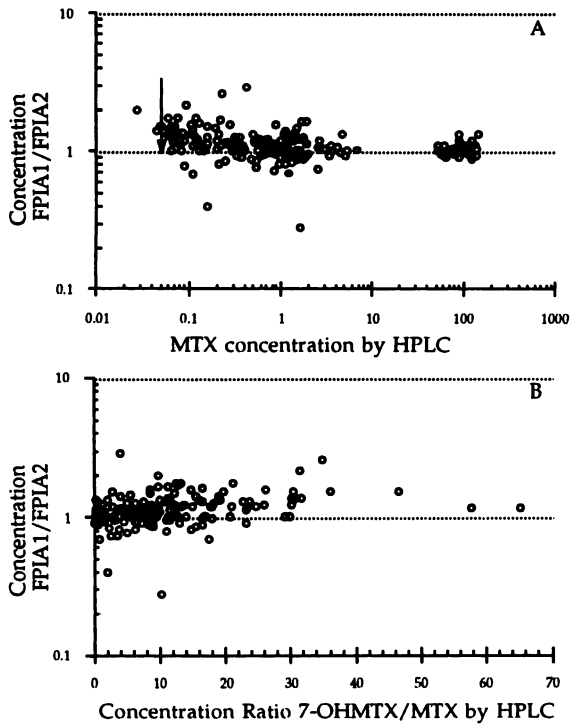

Fig. 4. Relation between ratios of plasma MTX concentrations measured by FPIA1 and FPIA2 to HPLC (*A*) and influence of 7-OHMTX on MTX analysis (*B*).

Arrow shows the limit of quantification.

limit of quantification of the Emit assay is ~6 and 30 times higher than that of FPIA and EIA, respectively. Because MTX inhibits DNA synthesis at concentrations <10 nmol/L *[23]*, it is an advantage to monitor MTX concentrations as low as this.

The within- and between-run precisions of Emit, FPIA1, and FPIA2 assays are in agreement with those reported by the respective manufacturers. For measuring MTX, FPIA1 and FPIA2 closely agreed regarding cross-reactivity with 7-OHMTX in samples with a low 7-OHMTX/MTX ratio. In contrast, in samples with a high 7-OHMTX/MTX ratio, FPIA2 gave a more accurate value—confirming the statements of the manufacturer that the monoclonal antibody in the new kit was less cross-reactive.

The HPLC assays are generally specific and sensitive for substances to be analyzed, but we found greater inter- and intraassay variabilities for HPLC than for the automated methods. HPLC assays are also time and labor consuming, and their utility in clinical settings may be limited. HPLC and EIA require more plasma volume than Emit and FPIA; this can be a problem in pediatric patients if capillary sampling is to be used.

In conclusion, the present study shows that all automated methods in the clinical laboratory are subject to cross-reactivity with plasma metabolites or endogenous substances. The discrepancy may result from cross-reactivity of antibodies with MTX metabolites in Emit and FPIA. EIA underestimated the MTX measurement at low concentrations but showed the best performance in comparison with HPLC. The CVs for both within- and between-run precision were considered to be within the performance limits required for monitoring MTX in all the methods examined. We conclude that the results of MTX measurement should be interpreted with caution, considering that they might influence the leucovorin administration and subsequently the therapeutic effectiveness as well as the risk of toxicity after high-dose MTX. In particular, when designing high-dose MTX protocols to be used at different centers, we think the method of MTX determination should be standardized.

Table 3. Variance[a] of immunoassay results from HPLC results in 42 courses of leucovorin treatment.

	Hours after MTX infusion				
	42		66		
Assay	+	−	+	−	No. of misestimated courses (% of total courses)
EIA	1	5	0	2	8 (19)
Emit	5	2	3	2	12 (29)
FPIA1	0	4	2	0	6 (14)
FPIA2	0	3	0	1	4 (10)

[a] Number of courses with overestimated (+) and underestimated (−) leucovorin doses.

Supported by grants from the Swedish Children Cancer Society and the Jenny Nordqvist Foundation.

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Leukoencephalopathy Following High-Dose Iv Methotrexate Chemotherapy With Leucovorin Rescue[1,2,3]

Jeffrey C. Allen,* Gerald Rosen, Bipin M. Mehta, and Bruce Horten[4]

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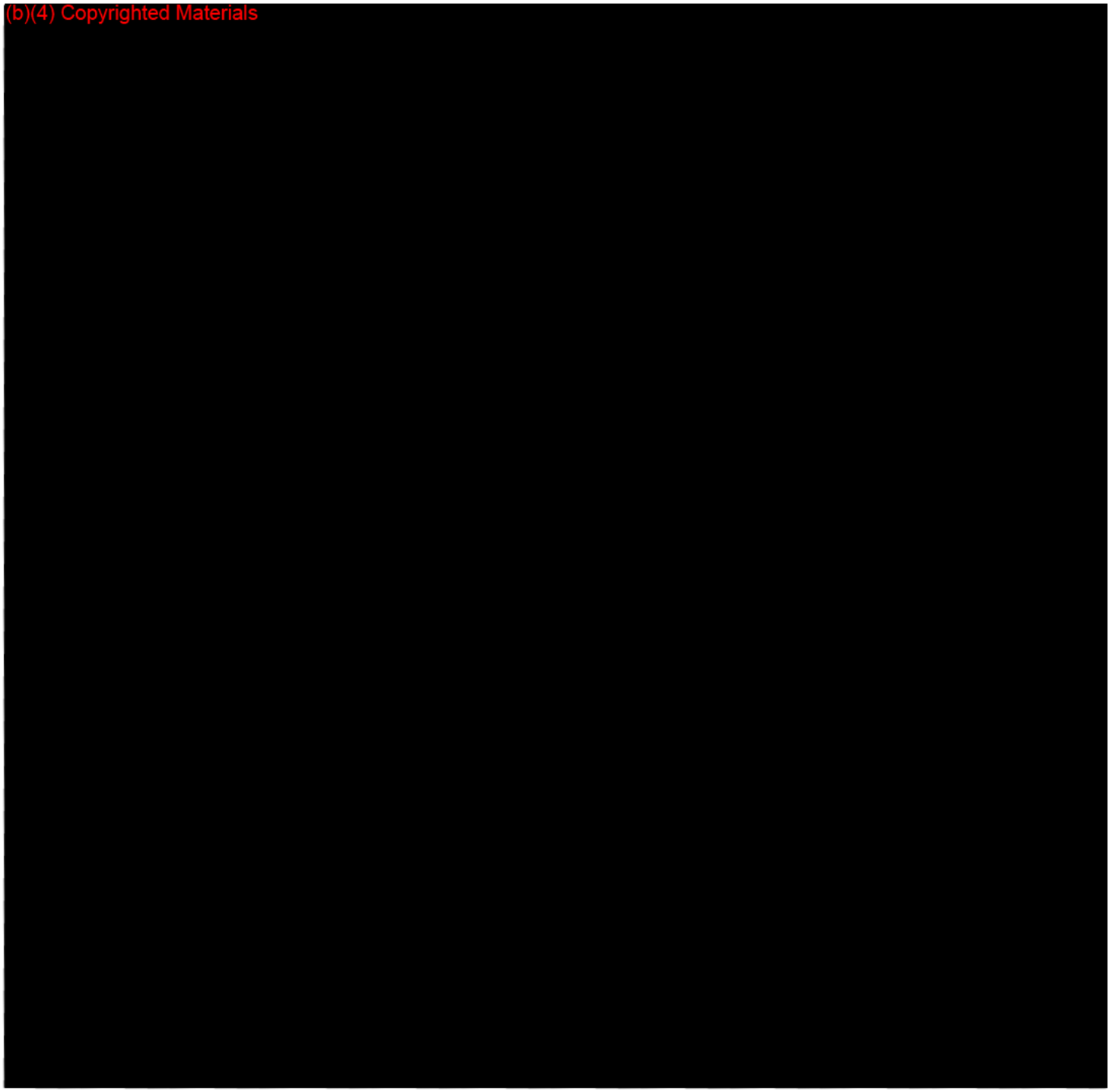

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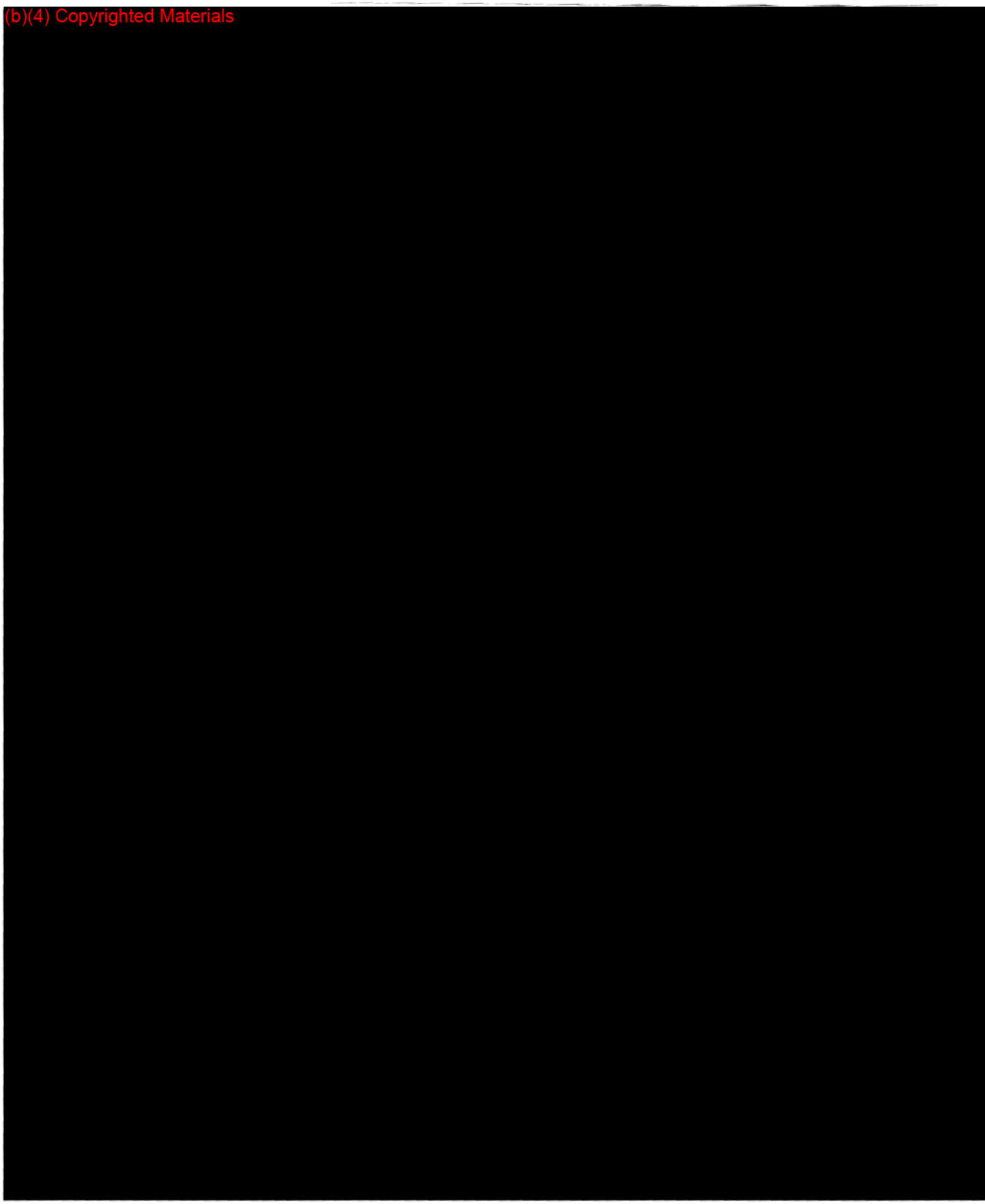

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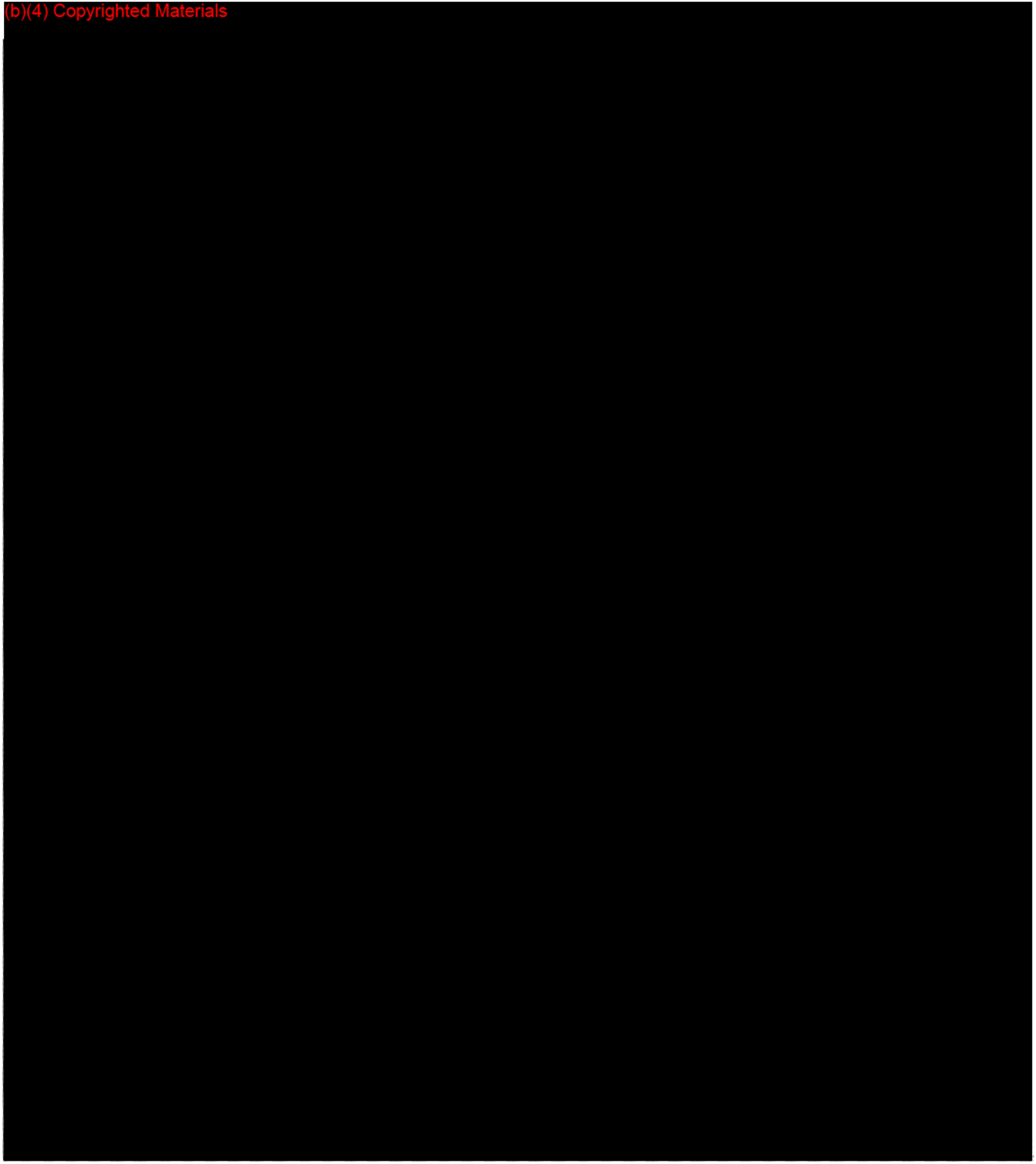

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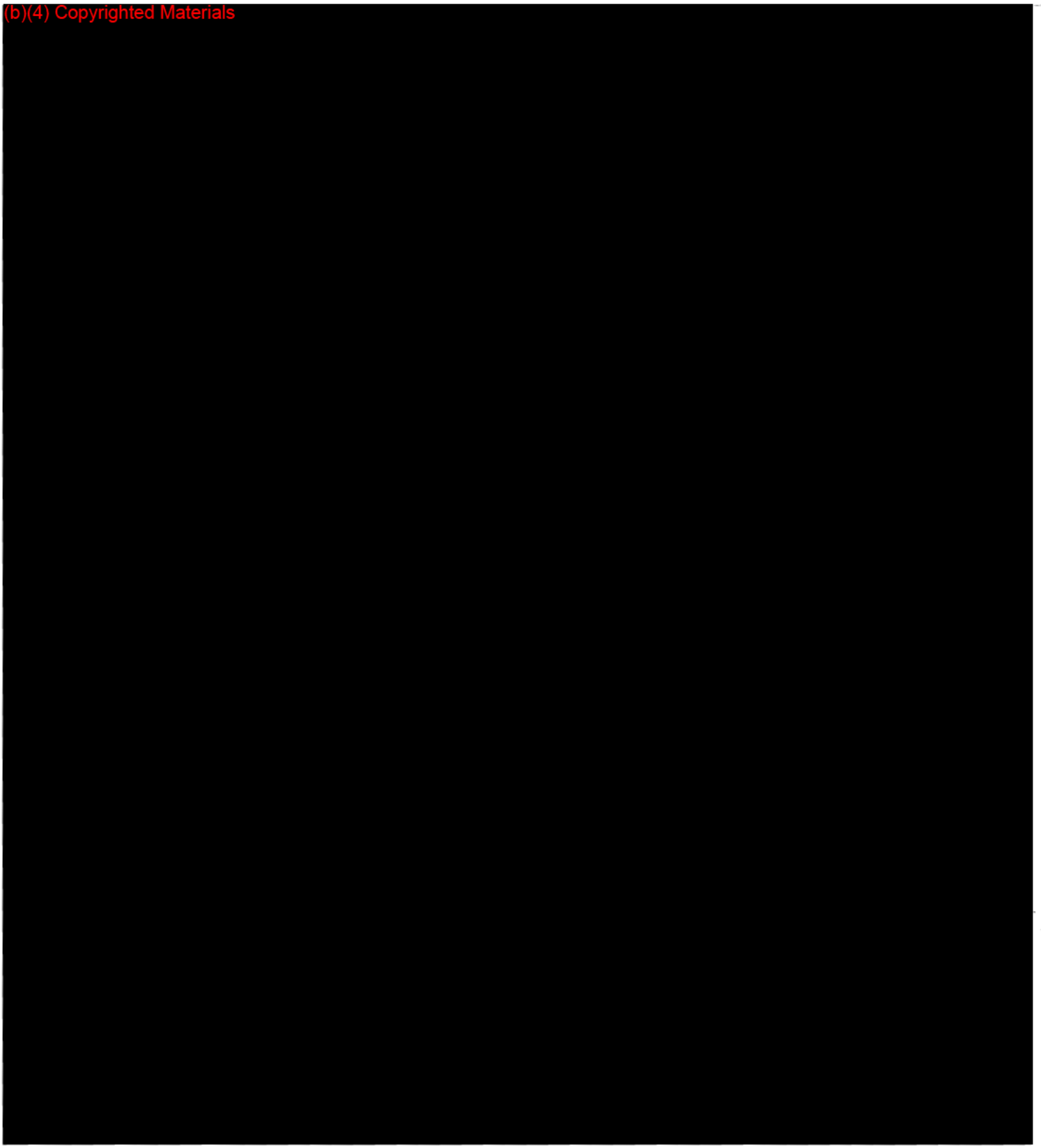

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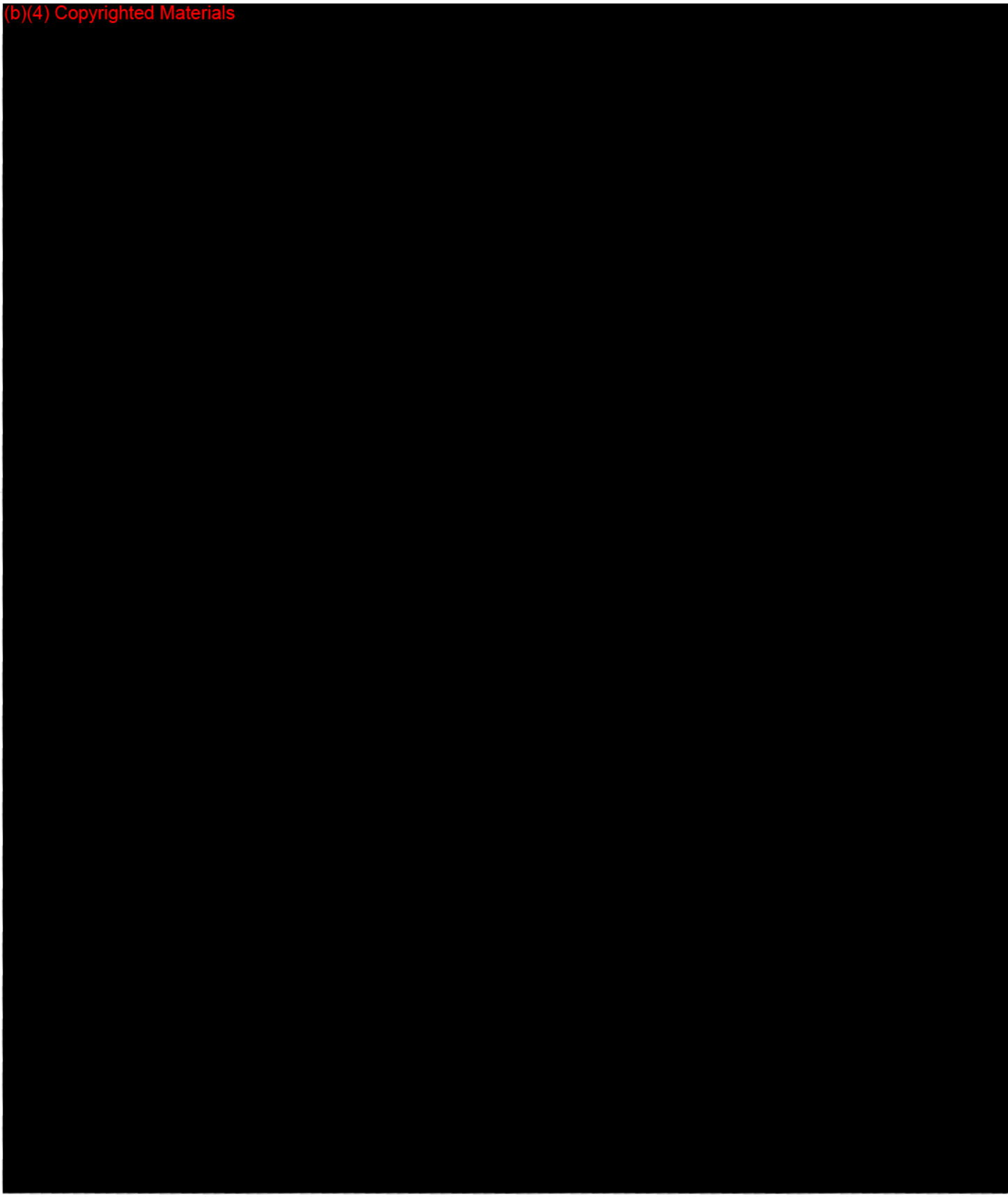

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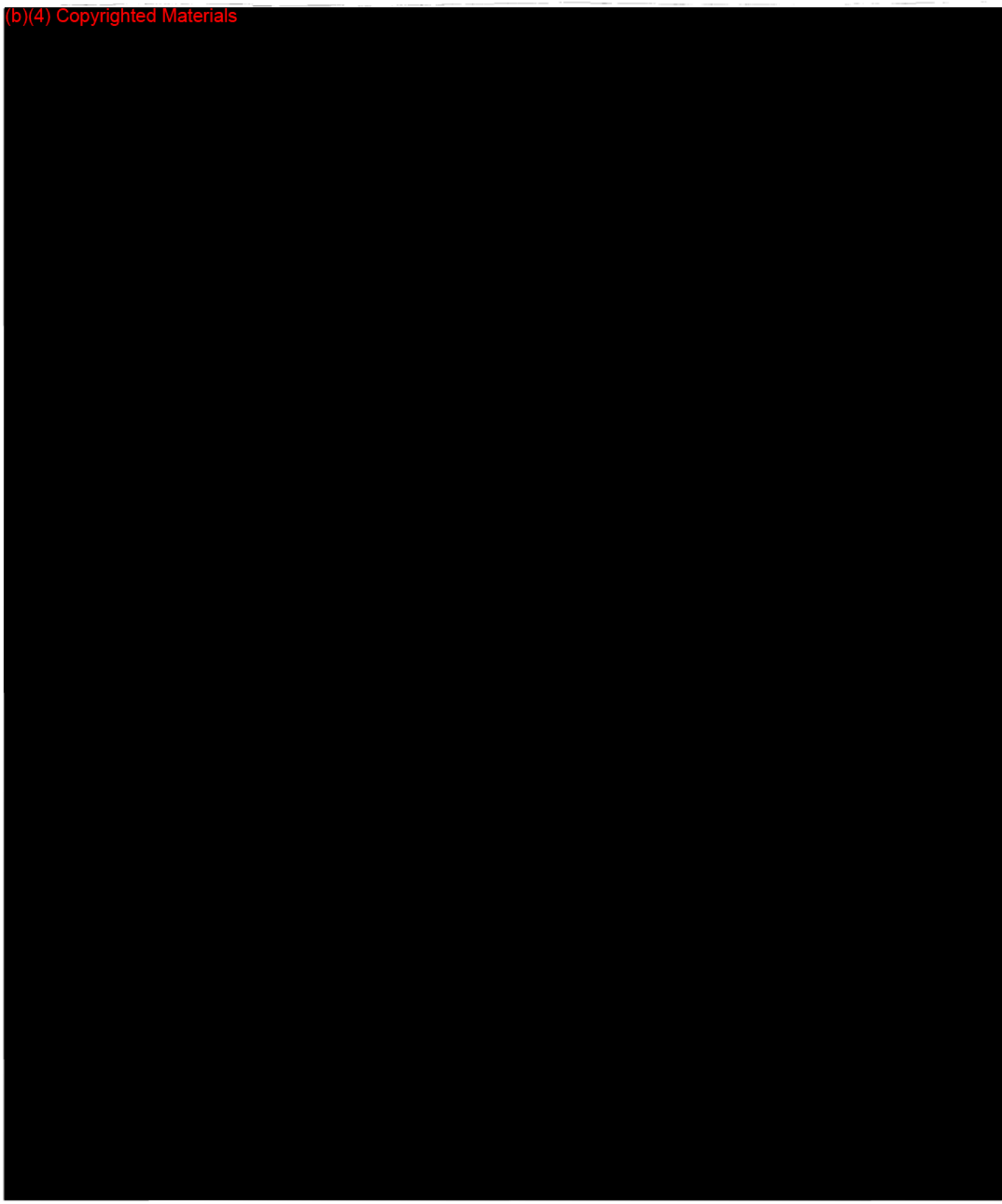

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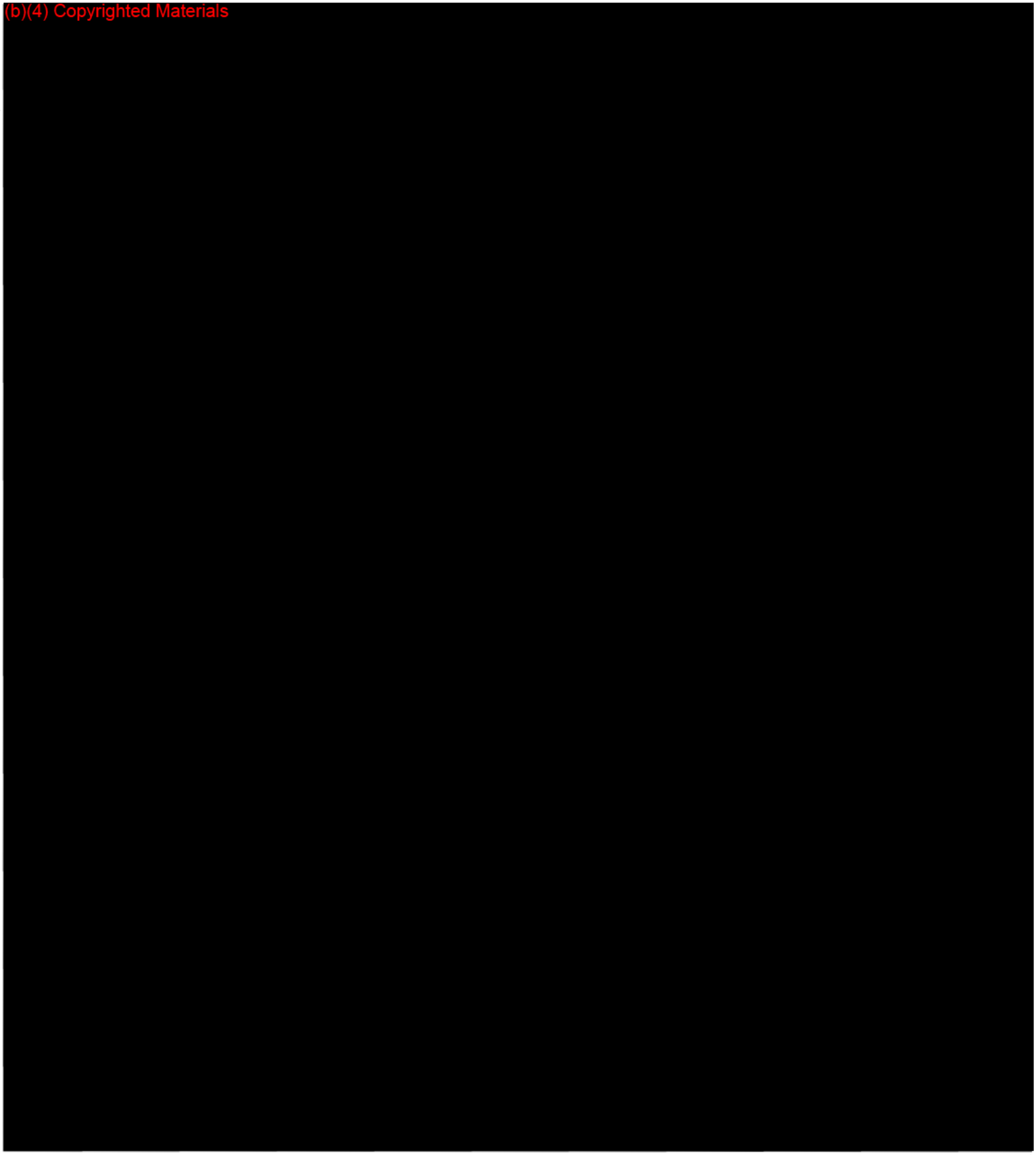

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CLIN. CHEM. 37/3, 398–402 (1991)

Determination of Product Shelf Life and Activation Energy for Five Drugs of Abuse

Geoffrey Anderson and Milda Scott

Estimating the shelf life of reagents and controls is a critical step in evaluating new formulations. We describe several stability assessment techniques, giving particular attention to experimental design. Activation energy was experimentally determined for five major drugs of abuse and metabolites: morphine, 11-nor-Δ-9-tetrahydrocannabinol-9-carboxylic acid, amphetamine, phencyclidine, and benzoylecgonine. The use of activation energy in establishing shelf life is illustrated for SENTRY™ (Hycor Biomedical Inc.) Drugs of Abuse Screen Control, a liquid urine-based product.

Additional Keyphrases: *Arrhenius plots · accelerated stability testing · control materials · bracket table · Q Rule · activation energy*

The shelf life of a product may be defined as the time that essential performance characteristics are maintained under specific handling conditions. Although it may be estimated by accelerated stability testing protocols, real-time product stability testing is necessary to validate stability claims for clinical chemistry reagents and reference material. Manufacturers' quality-assurance criteria typically require that a product must recover at least 90% of the initial value throughout its life. Applying this performance criterion to the results of stability testing thereby determines shelf life. However, alternative criteria are sometimes advisable.

Theory

Real-Time Stability Testing

In real-time stability testing, the duration of the test period should be long enough to allow significant product degradation under recommended storage conditions. At the least, the testing protocol must permit one to distinguish percent degradation from interassay variation. For example, data may be collected at an appropriate frequency such that a trend analysis may discern instability from day-to-day imprecision. The reliability of data interpretation can be increased by including in each assay a single lot of reference material with established stability characteristics. Sample recovery between assays can be thereby normalized to this reference, minimizing the impact of systematic drift and interassay imprecision. Frequently, however, an appropriate reference material is not available for use as a control.

When one measures the stability of a reference material, imprecision may be introduced by changes in both reagents and instrumentation. Ideally, reagents should be sufficiently stable that a single lot provides unchanging performance throughout the stability study and instrument performance should remain constant. However, one must monitor system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation.

Accelerated Stability Testing

Accelerated stability testing is often used in the development of clinical reagents to provide an early indication of product shelf life and thereby shorten the development schedule. To this end, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This information is then projected to predict product shelf life or used to compare the relative stability of alternative formulations.

A reasonable statistical treatment in accelerated stability projections requires that at least four stress temperatures be used. In addition, more nearly accurate stability projections are obtained when denaturing stress temperatures are avoided. This finding is particularly true for reagents containing labile, proteinaceous components.

Accelerated stability testing protocols allow one to stress samples, refrigerate them after the stressing, and then assay them simultaneously. Because the duration of the analysis is short, the likelihood of instability in the measurement system is reduced. Accelerated stability protocols additionally permit one to compare the unstressed product with stressed material within the same assay, the stressed sample recovery being expressed as a percent of unstressed recovery. This utilization of product as an internal control is especially valuable when no suitably stable reference material is available (*1*).

The Arrhenius Equation and Activation Energy

Most accelerated testing models are based on the Arrhenius equation (*2*):

$$\log\left(\frac{k_2}{k_1}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right)$$

where k_2 and k_1 are rate constants at temperature T_2 and T_1, respectively; E_a is the activation energy; and R is the gas constant. Temperature is in kelvins.

This equation describes the relationship between storage temperature and degradation rate. Use of the Arrhenius equation permits a projection of stability from the degradation rates observed at high temperatures (*3*). Activation energy, the independent variable in the

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Received June 8, 1990; accepted January 9, 1991.

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equation, is equal to the energy barrier that must be exceeded for the degradation reaction to occur. When the activation energy is known (or assumed), the degradation rate at low temperatures may be projected from those observed at "stress" temperatures.

The relationship between drug concentration [D] after time (t) for a first-order equation is

$$\frac{-d[D]}{dt} = K_1 [D]$$

where K_1 is the reaction rate constant at a given temperature (*2*). Integrating from $t = 0$ to $t = t$, one obtains

$$\ln[D] = \ln[D]_0 - K_1 t$$

$$\text{or } \log[D] = \log[D]_0 - (K_1 t/2.303)$$

where $[D]_0$ is the drug concentration at time 0. By substituting $0.90\ [D]_0 = [D]$ in the equation above, the time required to reach 90% of the original drug concentration can be shown to be

$$t_{90} = 0.105/K_1$$

A common practice of manufacturers in pharmaceutical and diagnostic reagent industries is to utilize various shortcuts, e.g., bracket tables (*4*) and the Q Rule (*2*), to estimate product shelf life. These techniques (see below) share the advantage that decisions may be made by analyzing only a few stressed samples. However, they are based on assumptions about the activation energy of product components and are valid only insofar as these assumptions are accurate. Whatever method is chosen, the validity of product stability projections depends on analytical precision, the use of appropriate controls within the experimental design, the assumptions embodied in the mathematical model, and the assumed or measured activation energy of product components.

Reaction rate is influenced by pH, tonicity, the presence of stabilizers, and so forth. Key product component(s) may degrade (or otherwise become unavailable) through multiple mechanisms (*2, 3*). In complex chemical systems, therefore, minor variation in formulation can profoundly affect lot-to-lot stability and, indeed, the activation energy of product degradation. Consequently, shelf life projected from accelerated studies must be validated by appropriate real-time stability testing.

Rapid Techniques for Projecting Shelf Life

Examples of shortcuts for projecting product shelf life for storage at 5 °C include the Q Rule and bracket table methods.

Q Rule. The Q Rule states that a product degradation rate decreases by a constant factor (Q_{10}) when the storage temperature is lowered by 10 °C. The value of Q_{10} is typically set at either 2, 3, or 4 because these correspond to reasonable activation energies. For larger shifts in temperature, the rate constant changes exponentially with temperature, and is proportional to $(Q_{10})^n$, where n equals the temperature change (°C) divided by 10. For example, the estimated decrease in degradation rate caused by lowering the storage temperature by 50 °C is $(Q_{10})^5$ because $n = 50/10 = 5$ (see Table 1). This model falsely assumes that the value of Q does not vary with temperature. A more detailed treatment has been published (*2*).

A Q_{10} value of 2 provides a conservative estimate, and results calculated with this value are considered *probable*. A Q_{10} value of 4 is less conservative and yields results considered to be *possible*.

To illustrate the application of the Q Rule in predicting shelf life, assume that 90% of phencyclidine (PCP) is recovered after 26 days at 55 °C. The stability of PCP under refrigerated (5 °C) conditions may be estimated [26 days/$(Q_{10})^5$] as follows:

(*a*) PCP is *probably* stable for 832 days (2.3 years) if $Q_{10} = 2$.

(*b*) PCP *may be* stable for 6318 days (17 years) if $Q_{10} = 3$.

(*c*) PCP is *possibly* stable for 26 624 days (73 years) if $Q_{10} = 4$.

Bracket tables. The bracket table technique assumes that, for a given analyte, the activation energy is between two limits, e.g., between 10 and 20 kcal. As a result, a table may be constructed showing "days of stress" at various stress temperatures (Table 2). The use of a 10 to 20 kcal bracket table is reasonable, because broad experience indicates that most analytes and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range (*4, 5*).

Because the bracket table provided in Table 2 does not specify stability requirements at a stress temperature of

Table 1. Q_{10} Factors

Q_{10}	E_a, kcal/mol	$(Q_{10})^5 = Q_{50}$
2.0	12.2	32
3.0	19.4	243
4.0	24.5	1024

Table 2. Bracket Table[a]

	Days of stress to predict stability at 5 °C for							
	6 months		1 year		2 years		3 years	
Stress temp., °C	20 kcal	10 kcal	20 kcal	10 kcal	20 kcal	10 kcal	20 kcal	10 kcal
14.5	55.3	100	111	201	221	402	332	603
25	16.1	54	32	108	64	217	97	326
35.5	5.1	30.6	10	61	20	122	31	183
47.5	1.5	16.6	3	32	6	66	9	100
60	0.5	9.2	0.9	18	1.9	37	2.8	55

[a] To use this table, select the column appropriate to the stability claim to be evaluated, e.g., 1 year. Acceptable performance after the number of days of stress in the 20-kcal column predicts that it is *possible* the selected claim will be observed (e.g., stressed for 32 days at 25 °C). Acceptable performance indicated in the 10-kcal column predicts that it is *probable* the selected claim will be observed.

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55 °C, a conservative use of the table requires that projections be taken from the 47.5 °C data. The PCP stability assumed in the Q Rule illustration (26 days) exceeds the six-month stability requirement (16.6 days) for the 10 kcal model. Thus, the interpretation of the bracket table is that PCP is *probably* stable at 5 °C for at least six months. Furthermore, because the assumed 26 days also exceeds the nine days stability required for the three-year 20 kcal model, it is *possible* that PCP is stable for at least three years.

Prudent use of either of these rapid techniques would dictate that data at three or four higher temperatures be incorporated into the projection of refrigerated shelf life. To evaluate the usefulness of the Q Rule and the bracket table in this example, one must determine the activation energy for PCP and then project the refrigerated shelf life by using the Arrhenius equation.

Materials and Methods

The activation energies of the five drugs of abuse in the SENTRY™ (Hycor Biomedical Inc., Garden Grove, CA 92641) liquid urine control were determined in three steps. First, we stressed the samples at high temperatures and determined the recovery of each drug. Rate constants were then derived by plotting analyte recovery vs days of heat stress at each temperature. Finally, we calculated the activation energy of each drug, using the Arrhenius equation.

Individual vials were stressed in constant-temperature incubators under the conditions summarized in Table 3. Semi-quantitative drug concentrations of each stressed sample were measured with the TD_x™ system (Abbott Labs., North Chicago, IL 60064), with fluorescence polarization immunoassay reagents. The manufacturer's recommendations were followed for instrument operation and maintenance. TD_x controls were assayed and the acceptability of each run was determined by comparing the recovery of the control with the manufacturer's stated acceptance range.

Because neither the 5 °C nor the −15 °C control values changed significantly with time, we used the 5 °C control results as "100% recovery" in each run. That is, drug recovery in stressed samples was expressed as a percent of that recovered in the 5 °C control. For each stress temperature, relative recovery values were plotted against days of heat input. The slope of the best-fit line was taken as the degradation rate constant, k.

The activation energy, E_a, for the chemical degradation of each drug was calculated from the Arrhenius equation by inserting the corresponding degradation rate constants.

Results

The percent drug recovery relative to that of the 5 °C control was calculated for samples at each stress condition; these data were analyzed by regression analysis to determine the slope of the best-fit line (Figure 1). These slopes, equal to the rate constants, were used to calculate the activation energies by use of the Arrhenius equation (Table 4).

Figure 2 illustrates for each drug the observed relationship between degradation rate and stress temperature. Note that 278 K is equivalent to 5 °C; the inverse

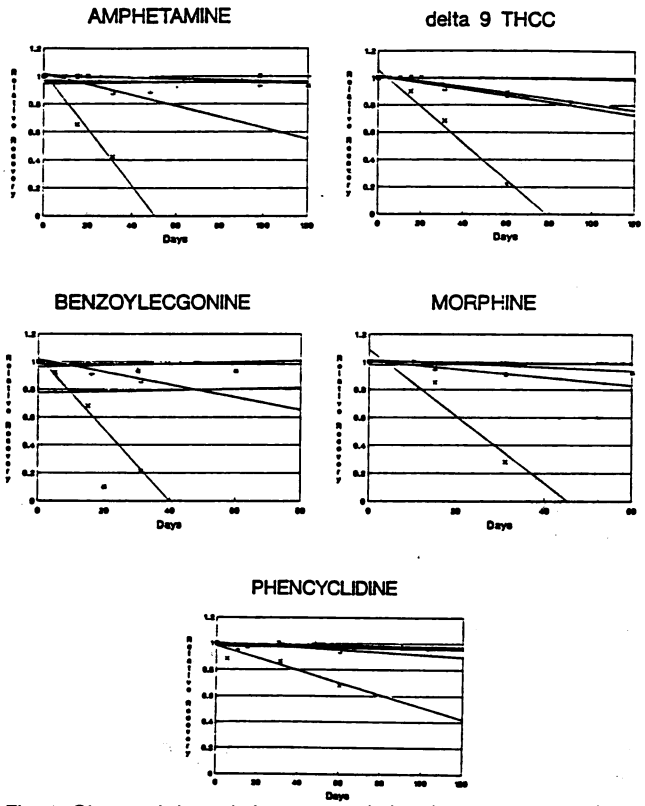

Fig. 1. Observed degradation rates: relative drug recovery vs time
The rate of drug loss at a given temperature is equal to the degradation rate constant, k. The dependence of this constant on temperature is illustrated for each drug by the slope of each line: ●, 15 °C; +, 25 °C; *, 35.5 °C; □, 45 °C; X, 55 °C; delta 9 THCC, 11-nor-Δ-9-tetrahydrocannibinol-9-carboxylic acid

Table 3. Accelerated Stress Conditions Used to Calculate Degradation Rate Constants for the Drugs of Abuse Study

Temp., °C	Days of stress								
−15	5	10	15	30	48	60	90	120
5	5	10	15	30	48	60	90	120
15				30		60	90	120
25			15	30		60	90	120
35		10		20			90	120
45	5		15	30				
55	5		15	30				

Table 4. Calculated Degradation Rate Constants and Activation Energies

Drug	Rate constants, $k \times 10^3$					E_a,
	15 °C	25 °C	35 °C	45 °C	55 °C	kcal/mol
Morphine	0.20	0.30	1.3	2.7	22	30
Delta-9-THCC[a]	0.01	0.11	0.20	2.2	34	35
Amphetamine	0.25	0.63	0.42	3.4	19	24
Phencyclidine	0.20	0.40	0.50	1.0	5.3	18
Benzoylecgonine	0.02	0.02	2.2	3.7	25	33

[a] See legend to Fig. 1.

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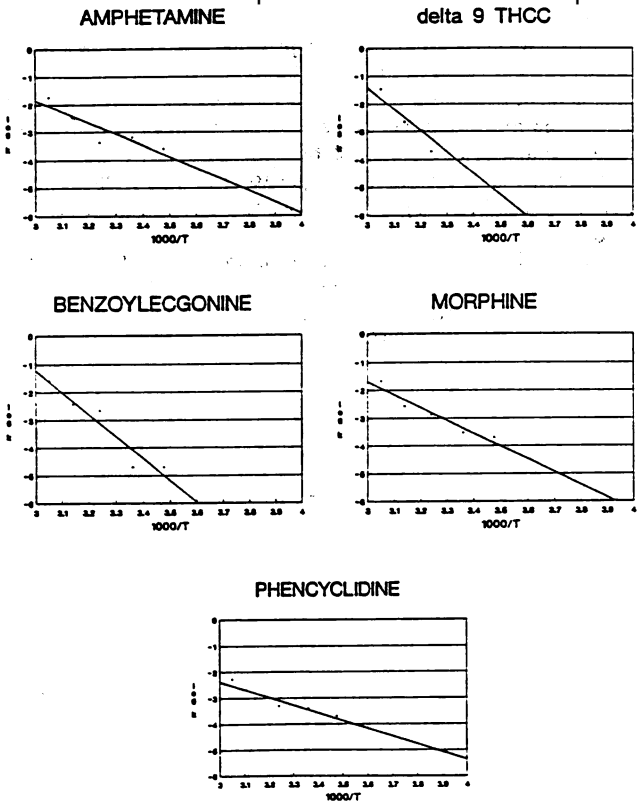

Fig. 2. Arrhenius plots

The log *k* values derived in this study are plotted vs inverse temperature (K). Projected values of *k* at 5 °C (3.5 on the *abscissa*) are useful in determining drug stability at refrigerated temperatures

of this absolute temperature is "3.6" on the Arrhenius plot. The Arrhenius plots obtained are approximately linear for each drug. We used regression analysis to project the 5 °C rate constant for each drug. The period over which at least 90% of the initial drug is recovered was calculated from the equation: stability (days) = 0.105/*k*. Finally, the calculated stabilities were supported by the observed 18-month recovery of these drugs in the SENTRY Control maintained at 5 °C (Table 5).

Discussion

The projected stability of a refrigerated product is determined exclusively by the calculated 5 °C degradation rate constant. This projected stability depends on the activation energy and cannot reliably be projected by monitoring drug recovery at a single stress temperature.

In the present study, we used simple linear-regression analysis to fit experimental data. Alternatively, Arrhenius analysis can be fine-tuned by using estimates of analytical error to establish a confidence interval for each rate constant (*2, 3*). This analysis permits a *weighted* least-squares statistical treatment and produces a more statistically valid projection of product stability at lower temperatures. One can calculate the fit of observed rate constants to the Arrhenius relationship and thereby evaluate the validity of the accelerated stability study (*6*).

As shown in Table 4, amphetamine has the highest observed degradation rates at 15 and 25 °C. At 35, 45, and 55 °C, benzoylecgonine has the highest rates. However, the 5 °C degradation rate constants calculated from the Arrhenius equation demonstrate that PCP has the highest rate constant, and is therefore the least stable under refrigerated conditions. The calculated activation energy for morphine is in fair agreement with a published value (*5*) of 23 kcal/mol. As previously mentioned, the reaction rate, and hence the activation energy, can be influenced by the chemical matrix in which drug stability is tested.

The quality-assurance risk inherent in using any method for stability estimation, including the Q Rule or bracket tables, is that one might assume the activation energy to be greater than it actually is. The stability in this case will likely be overestimated. On the other hand, if one assumes an activation energy lower than actual, the estimated stability may be so "conservative" that it is of little practical value.

Recall the assumed PCP stability in our example examination of the Q Rule and bracket table. This 26-day stability interval is in fact the experimentally observed stability of PCP in the current study. The activation energy of PCP, as well as its projected stability is based on data obtained at five stressing temperatures; the projected 5 °C stability of PCP is 10 years (Table 5).

The Q Rule provides a conservative estimate (2.3 years) if $Q_{10} = 2$. The estimated stability (17 years) is significantly higher, if $Q_{10} = 3$, despite the fact this Q_{10} value corresponds to an activation energy (19.4 kcal/mol, Table 1) near the calculated value (18 kcal/mol, Table 4). This estimation error likely results from the erroneous assumption that Q does not vary with temperature.

The bracket table method provides a good but conservative estimate of stability for PCP, the stability estimate from the 20 kcal column being at least three years.

In summary, accelerated stability testing and the various models available for interpreting these data can provide valuable information for evaluating reagents and control products. By optimizing the analytical precision and other aspects of test protocol design, one can

Table 5. Calculated and Observed Stability

Drug	Projected Stability, years	Observed recovery, %[a]
Morphine	57	92
Delta-9-THCC[c]	364	101
Amphetamine	18	100[b]
Phencyclidine	10	97
Benzoylecgonine	114	103

[a] After 18 months at 5 °C.

[b] 100% recovery was observed after six months at 5 °C; subsequent data showed an increased recovery owing to a specificity change in TD_x reagents for amphetamine.

[c] See legend to Fig. 1.

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expect both real-time and accelerated stability studies to provide more valid information. For analytes with high activation energies, such as the drugs of abuse studied here, both bracket tables and the Q Rule provide useful information when they are applied conservatively. Use of published or experimentally derived activation energy values can significantly lower the risks inherent in projecting product shelf life.

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Journal of Chromatography B, 661 (1994) 109–118

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

High-performance liquid chromatographic determination of methotrexate, 7-hydroxymethotrexate, 5-methyltetrahydrofolic acid and folinic acid in serum and cerebrospinal fluid

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First received 28 January 1994; revised manuscript received 27 June 1994

Abstract

A method for the simultaneous determination of the antifolates methotrexate and 7-hydroxymethotrexate as well as the folates 5-methyltetrahydrofolic acid and folinic acid (5-formyltetrahydrofolic acid) in serum and cerebrospinal fluid (CSF) is described. High-performance liquid chromatography with gradient elution and dual detection (ultraviolet absorption and fluorescence) was used to separate and quantitate the analytes. Serum samples containing high levels of the substances of interest and CSF samples were injected directly onto the HPLC column. For determination of low concentrations, serum samples were subjected to a solid-phase extraction method for clean-up and concentration purposes. The determination limits were 10 ng/ml for both antifolates, 100 ng/ml for folinic acid, and 0.1 ng/ml for the physiologically occurring methylated folate which is about 1/100 the serum concentration in healthy children. The suitability of the method for pharmacokinetic monitoring of high-dose methotrexate therapy combined with leucovorin rescue administered to children with acute lymphoblastic leukemia was demonstrated. Minimum values of the serum folate during treatment ranged from 0.2 to 3.1 ng/ml. Even those very low concentrations could be reliably measured.

1. Introduction

The cytostatic drug methotrexate (MTX, amethopterin, 4-amino-10-methylpteroyl-L-glutamic acid, see Fig. 1) acts as an antimetabolite and is widely used in the therapy of human malignancies. As an analog of folic acid (pteroylglutamic acid, PteGlu) MTX inhibits the enzyme dihydrofolate reductase causing a lack of reduced folates which are donors of one-carbon units in the biosynthesis of nucleic acids and some amino acids. Like the folates, MTX is intracellularly converted to polyglutamates, which inhibit further enzymes of the folate metabolism, and thus take part in the cytostatic action of the drug [1,2].

MTX is metabolized in the liver to 7-hydroxymethotrexate (7-OH-MTX), which is about 200-fold less effective as inhibitor of dihydrofolate reductase than the parent compound [3]. On the

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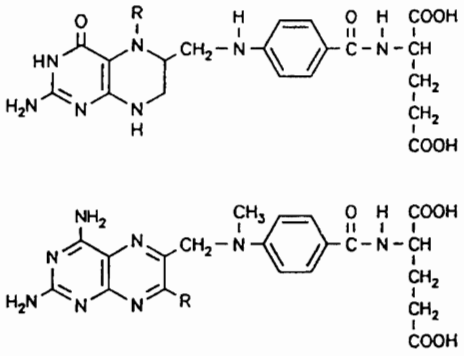

Fig. 1. Chemical structures of (A) 5-CH_3-H_4PteGlu (R = CH_3), 5-CHO-H_4PteGlu (R = CHO), (B) MTX (R = H), 7-OH-MTX (R = OH).

other hand, polyglutamates of the metabolite are highly cytotoxic [4,5].

High-dose MTX therapy must be combined with administration of reduced folates to prevent severe toxicity in the patients. For this purpose, leucovorin, the calcium salt of folinic acid ([6*R*,*S*]-5-formyltetrahydrofolic acid, 5-CHO-H_4PteGlu), is used. Its 6*S*-isomer is rapidly converted to [6*S*]-5-methyltetrahydrofolic acid (5-CH_3-H_4PteGlu) which is the transport form of the folates and occurs physiologically in serum and cerebrospinal fluid (CSF). The serum levels of healthy individuals are 5.8 ± 2.8 ng/ml for adults and 2–20 ng/ml for children (1–15 years), respectively [6–8]. The concentration in CSF is two to four times that of blood serum [6,9]. The 6*R*-isomer of 5-CHO-H_4PteGlu is eliminated in a non-metabolized form by the kidneys [10].

Although the pharmacokinetics of MTX has been extensively studied [5,11], neither the optimal MTX dose nor the best leucovorin regimen have yet been established [12]. With better knowledge about the metabolization of the drug and the behaviour of folates during MTX and leucovorin administration, we expect to get better insight in the mechanisms of MTX and rescue efficacy as well as of toxicity. For this purpose, a method for the simultaneous monitoring of MTX, 7-OH-MTX, 5-CHO-H_4PteGlu and 5-CH_3-H_4PteGlu serum levels was required.

In the literature a number of methods are described that either analyze MTX (HPLC [13–15], immunoassays [16,17]) or folates (HPLC [18–20], microbiological [21,22] or radiometric assays [23]). Only few methods allow the simultaneous determination of folates and antifolates [12,24–26]. However, all of them show disadvantages: either determination of 7-OH-MTX is not possible [25,26], or the detection of 5-CH_3-H_4PteGlu is not sensitive enough to measure the expected low serum concentrations during therapy and even physiological levels cannot be determined [12,24]. To overcome these problems a sensitive HPLC method was developed with ultraviolet and fluorescence detection following a sample clean-up and concentration procedure using solid-phase extraction (SPE).

2. Experimental

2.1. Chemicals and reagents

The calcium salt of [6*R*,*S*]-5-methyl-5,6,7,8-tetrahydrofolic acid was obtained from Merck (Darmstadt, Germany), leucovorin ([6*R*,*S*]-5-formyl-5,6,7,8-tetrahydrofolic acid calcium salt) and methotrexate were supplied by Lederle (Wolfratshausen, Germany). Acetonitrile came from Baker (Gross-Gerau, Germany) and was of HPLC quality. All other chemicals obtained from Merck were of analytical grade.

In order to obtain standardized conditions for blank and spiked samples we used a commercially available serum protein solution (Serumar, Armour Pharma, Eschwege, Germany). The chromatogram of Serumar showed no interference with the peaks of interest and 5-CH_3-H_4PteGlu could not be detected.

7-OH-MTX standard solutions were prepared in 0.05 *M* NaOH. For other standards and for sample solutions we used a buffer with pH 7.6 made of 0.1 *M* Tris-HCl with 1% L-ascorbic acid and flushed with nitrogen to remove the oxygen (in the following referred to as Tris-HCl buffer). Stored at −20°C in the dark the standard solution of 5-CH_3-H_4PteGlu remained stable for about 6 months; all other standard solutions were stable for a period of 2 years.

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2.2. Preparation of 7-OH-MTX

The MTX metabolite 7-OH-MTX was prepared enzymatically by the method described by Cairnes and Evans [27], in which a rabbit liver homogenate was incubated with MTX. After 3 h the incubation mixture yielded 72% of 7-OH-MTX and 28% of MTX as measured by HPLC. The metabolite was subsequently recrystallized four times from acid solution and dried in a desiccator above phosphorus pentoxide. The resulting orange coloured powder contained 97% of 7-OH-MTX (as determined by HPLC).

2.3. Sample preparation

Blood samples were collected in non-heparinized tubes. After clotting at room temperature and centrifugation (5 min at 3000 *g*), the serum was separated and frozen until analysis. Samples of cerebrospinal fluid were taken by lumbar puncture and frozen. Samples were stored at −20°C for up to 7 days, thereafter at −80°C. No degradation was observed for spiked serum samples during a test period of 6 months. Before thawing, 5 mg of ascorbic acid was added per milliliter and the samples were thawed slowly at a temperature of about 4°C.

Storage and sample preparation were performed under protection from light. Serum samples containing more than 5 μg/ml of MTX or 7-OH-MTX were diluted five-fold with Tris-HCl buffer before direct injection. Other serum samples were cleaned up and concentrated with a solid-phase extraction method [12]. For this purpose two different buffers were used: Buffer A with a pH of 5.0 consisted of 0.1 *M* citric acid and 0.2 *M* disodium hydrogenphosphate, buffer B was a two-fold dilution of buffer A with distilled water. A 500-μl aliquot of each serum sample was mixed with 2.5 ml of buffer A and extracted on a C_{18} SPE column (200 mg Bond Elut, ICT, Frankfurt, Germany) that was pretreated with 5 ml of methanol and 3 ml of buffer B. After transferring the sample solution to the SPE column, the column was washed three times with 1 ml of buffer B, 0.5 ml of 20 m*M* NaOH and again with 0.5 ml of buffer B. The analytes were extracted with 1.0 ml of methanol. Then the methanol was evaporated at 60°C under a stream of nitrogen. The residue was redissolved in 100 μl of Tris-HCl buffer and the solution was mixed thoroughly. After membrane filtration (pore size 0.2 μm, Chromafil AO-20/3, Macherey-Nagel, Düren, Germany) the solutions were injected onto the HPLC column.

Cerebrospinal fluid samples were injected directly onto the HPLC column after membrane filtration.

2.4. High-performance liquid chromatography

The system consisted of a gradient pump (Model 480G, Gynkotek, Germering, Germany) with a degassing device (Shodex Degas, Showa Denko, Japan) and an automatic injector (GINA, Gynkotek). The injection volume was 20 μl. In order to obtain a better stability of the injected solutions the sample racks were cooled to 10°C using a cryostat (RMT 6, Lauda, Lauda-Königshofen, Germany). Two detectors were employed in series: a fluorescence spectrophotometer (F-1050, Merck/Hitachi, Darmstadt, Germany) with excitation and emission wavelengths of 295 and 355 nm, respectively, was used to detect low levels of 5-CH_3-H_4PteGlu (< 100 ng/ml in the injected solution). For the determination of MTX, 7-OH-MTX, folinic acid and high levels of 5-CH_3-H_4PteGlu, we used an ultraviolet detector (UVD 320, Gynkotek) operated at 310 nm. Data were processed by an interface (PE Nelson 900, Perkin Elmer, Überlingen, Germany) and a computing system. The separation was performed at room temperature on a Supelcosil LC-18-DB column (150 × 4.6 mm I.D., 3 μm), in connection with a precolumn (20 × 4.6 mm I.D.) packed with Supelcosil LC-18-DB, 5 μm. The mobile phase was a modification of that described by Hahn et al. [25]. It consisted of a gradient system using acetonitrile and phosphate buffer (5 m*M* KH_2PO_4, adjusted to pH 2.3 with phosphoric acid and filtered using a membrane with 0.45-μm pore size). The mobile phase components were mixed as mentioned in Table 1. The last step of the gradient was

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Table 1
Mobile phase composition

Time (min)	Solvent A[a] (%)	Solvent B[a] (%)
0	7	93
5	7	93
20	13	87
26	21	79
27	21	79
29	7	93

[a] A = acetonitrile, B = 5 m*M* KH_2PO_4 buffer, pH 2.3.

added to reequilibrate the column with the initial mobile phase. The flow-rate was 0.9 ml/min.

2.5. Calibration and recovery

For quantification, peak area integration with the external standard method was used. To determine the linear range and the detection limits, standard solutions with various concentration levels were chromatographed. The resulting calibration curves were used to quantitate the directly injected samples. For calibration of the samples subjected to the SPE we used the following method: a series of serum protein solutions spiked with standard mixtures of different concentrations was analyzed according to the method described here with SPE and HPLC. The resulting curves were then used to calculate the sample contents. The recovery was calculated by comparing the peak areas of the standard solutions and the spiked serum protein solutions.

3. Results

3.1. High-performance liquid chromatography

Fig. 2 shows the fluorescence and ultraviolet chromatograms of a standard mixture. In the fluorescence detector signal 5-CH_3-H_4PteGlu and 5-CHO-H_4PteGlu are visible, while the ultraviolet chromatograms show MTX, 7-OH-MTX and 5-CHO-H_4PteGlu. Methylated folate can also be detected with the ultraviolet detector

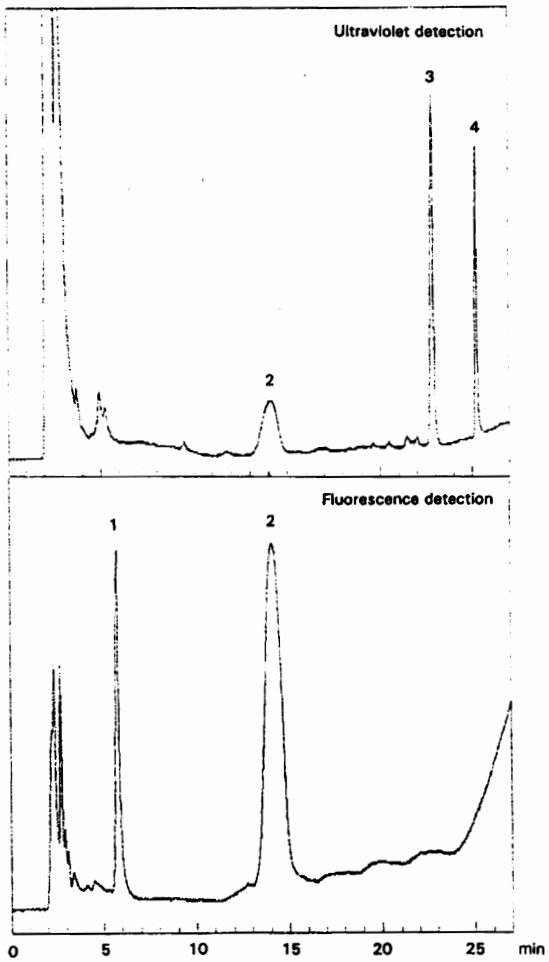

Fig. 2. Chromatograms of a standard mixture. Peak identification: 1 = 5-CH_3-H_4PteGlu, 2 = 5-CHO-H_4PteGlu, 3 = MTX, 4 = 7-OH-MTX. The absolute amounts injected were 0.4 ng of 5-CH_3-H_4PteGlu and 40 ng of each of the other substances. Chromatographic conditions see text.

when more than 2 ng are injected (see Fig. 4). HPLC of 5-CHO-H_4PteGlu gives an unusual broad but symmetric peak as can also be seen from chromatograms in other papers [12,19,24–26,28–30]. The separation is finished within 27 min and after an additional 5-min reequilibration time the next injection can be made.

The relative absorption maxima of the substances dissolved in mobile phase were at 295 nm for 5-CHO-H_4PteGlu and 5-CH_3-H_4PteGlu and at 310 nm for MTX and 7-OH-MTX, respectively. Because sensitive detection of the methylfo-

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late was performed using the fluorescence signal and the antifolates were of greater relevance than the formylfolate (see Discussion), 310 nm was chosen as detection wavelength.

Chromatograms of two serum samples and a CSF sample are shown in Figs. 3, 4 and 5, respectively. With the described gradient system the four substances of interest, MTX, 7-OH-MTX, 5-CH_3-H_4PteGlu and 5-CHO-H_4PteGlu, are well separated from endogenous peaks, at least in one of the detector signals. Analyzing samples taken from acute lymphoblastic leukemia (ALL) patients at different time points during therapy, we observed some additional

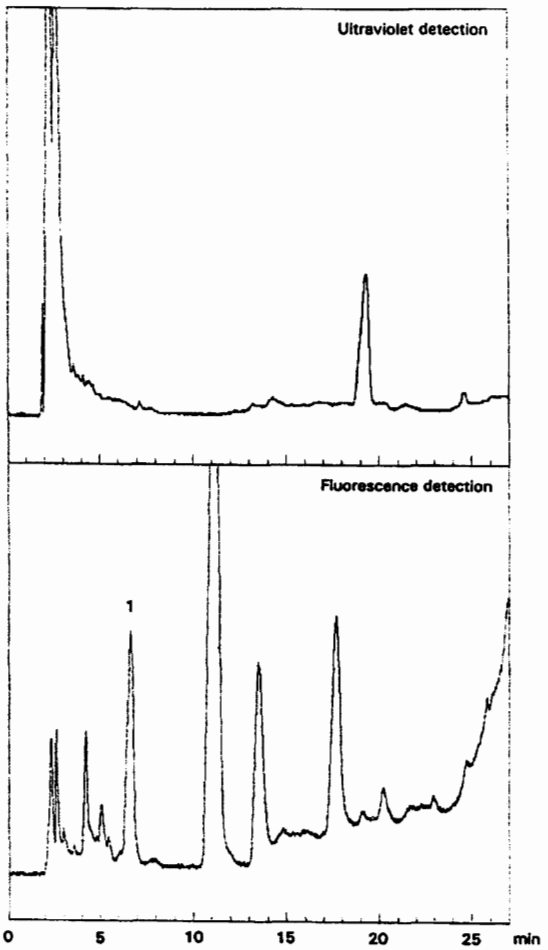

Fig. 3. Chromatograms of a serum sample from an ALL patient taken before start of therapy. Sample was analyzed using SPE. Chromatographic conditions see text, peak identification see Fig. 2. 5-CH_3-H_4PteGlu concentration was 6.1 ng/ml.

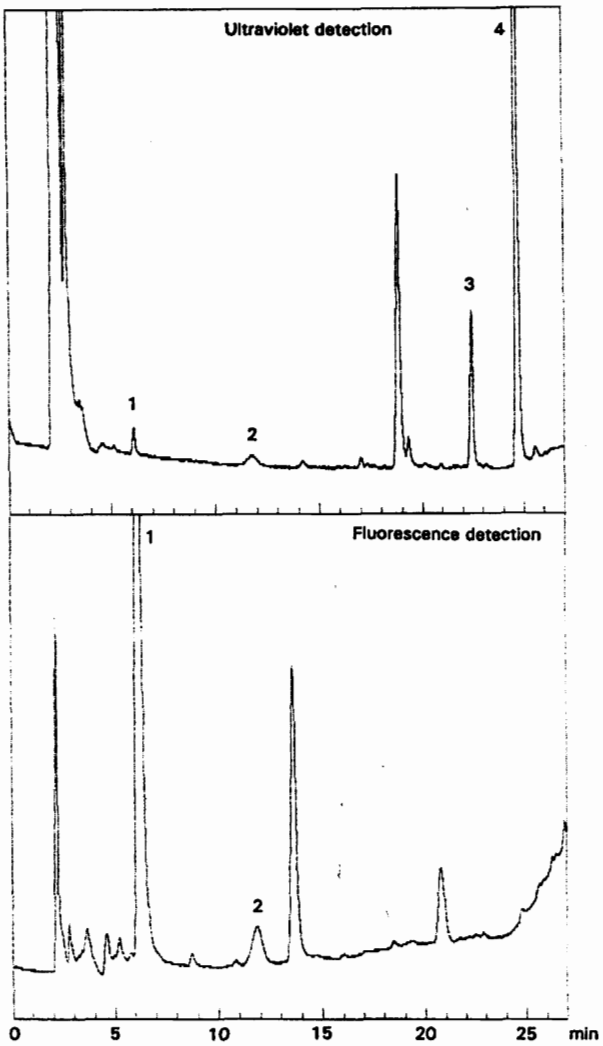

Fig. 4. Chromatograms of a serum sample from an ALL patient taken after MTX and leucovorin treatment. Sample was analyzed using SPE. Chromatographic conditions see text, peak identification see Fig. 2. Serum concentrations were 56.7 ng/ml of 5-CH_3-H_4PteGlu, 0.38 μg/ml of 5-CHO-H_4PteGlu, 0.19 μg/ml of MTX and 2.6 μg/ml of 7-OH-MTX.

peaks appearing in the chromatogram (not shown). That was why the chromatographic runtime could not be shortened without loss of separation. Since there was an unidentified peak with the same retention time as 5-CHO-H_4PteGlu, appearing in the fluorescence but not in the UV signal (see Figs. 3 and 5), we determined the formyl folate from the UV chromatogram.

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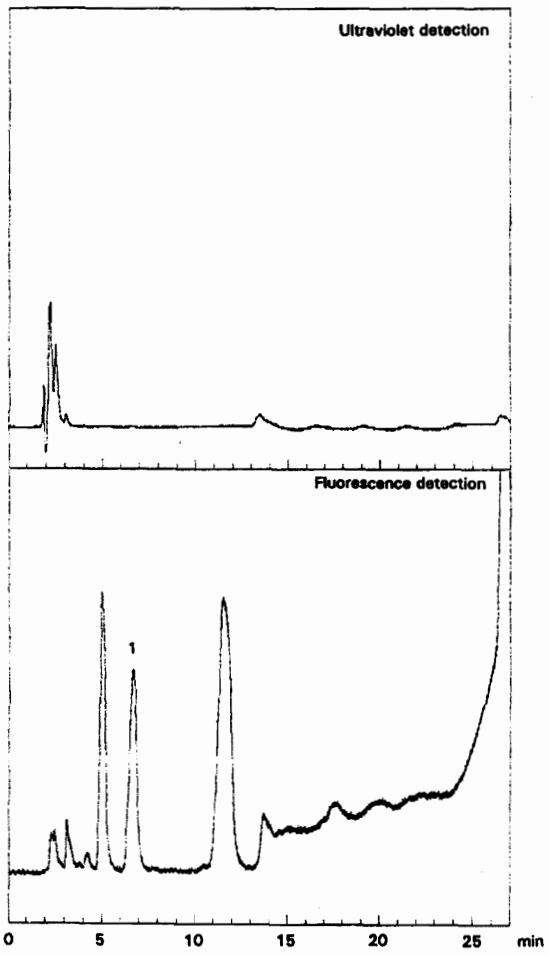

Fig. 5. Chromatograms of a CSF sample taken from an ALL patient before the intrathecal MTX application. Sample was directly injected onto the HPLC column. It contained 26.0 ng/ml of 5-CH_3-H_4PteGlu. Chromatographic conditions see text, peak identification see Fig. 2

3.2. Sample preparation

Because folates are labile substances, we performed recovery experiments with spiked serum protein solutions. It was shown that the thawing temperature influences the stability of 5-CH_3-H_4PteGlu and 5-CHO-H_4PteGlu. Maintaining a temperature of about 4°C while thawing the samples and addition of L-ascorbic acid were found to prevent loss of folates.

It is known that deproteinization of serum samples by heat treatment or trichloroacetic acid results in decomposition of the folates [19,30]. We observed that precipitation with perchloric acid even in the presence of ascorbic acid also caused high losses. Therefore, we used a SPE method to remove proteins and other interfering substances. Furthermore, there is also a concentrating effect. By using a 500-μl volume of serum or CSF and dissolving the residue of the SPE eluate in 100 μl of buffer we achieved a five-fold concentration.

At a temperature of 10°C in the dark, sufficient stability of the sample solutions during storage in the automated injector was achieved. Over a time period of 24 h we observed a loss of methyl-folate of ca. 5% and a less than 2% loss of the other substances.

3.3. Calibration and recovery

The curves of 5-CH_3-H_4PteGlu derived from analysis of the spiked serum protein solutions were linear over a concentration range of 0.1–100 ng/ml using fluorimetric detection and of 20–5000 ng/ml using the ultraviolet detector. 5-CHO-H_4PteGlu gave a linear range of 100–5000 ng/ml, MTX and 7-OH-MTX of 10-5000 ng/ml. The correlation coefficients were between 0.9942 and 0.9995 for MTX, 7-OH-MTX and 5-CH_3-H_4PteGlu, respectively; for 5-CHO-H_4PteGlu the correlation coefficient was 0.9889. The limits of determination are presented in Table 2. The precision of the sample preparation method was calculated by analyzing a spiked serum protein solution (1 μg/ml of each substance) five times in series on one day to get the intra-day variation (v_{id}) and on different days to get the day-to-day variation (v_{dd}), respectively. We found v_{id} values of 5% for 5-CH_3-H_4PteGlu and MTX, 7% for 7-OH-MTX and 23% for 5-CHO-H_4PteGlu. The v_{dd} values were 8% for 5-CH_3-H_4PteGlu, 2% for MTX and 7-OH-MTX, 34% for 5-CHO-H_4PteGlu.

The results of the recovery experiments are shown in Table 3. Using human serum we obtained similar results as with the commercial serum protein solution.

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Table 2
Limits of determination (calculated with a signal-to-noise ratio of 5)

Compound	Directly injected aqueous standard solutions		Spiked serum protein solutions injected after sample preparation	
	ng	pmol	ng/ml	μmol/l
5-CH_3-H_4PteGlu (FL)[a]	0.01	0.02	0.1	0.0002
5-CH_3-H_4PteGlu (UV)[a]	2	4.4	20	0.04
5-CHO-H_4PteGlu	2	4.2	100	0.2
MTX	1	2.2	10	0.04
7-OH-MTX	1	2.1	10	0.04

[a] Detection method: ultraviolet (UV) or fluorescence (FL).

3.4. Analyzed samples

A total of 220 serum samples was analyzed. Serum was taken from five children with ALL, each treated with four successive cycles consisting of high-dose MTX combined with leucovorin rescue. The observed concentration ranges are listed in Table 4. Before the start of the therapy the concentration of 5-CH_3-H_4PteGlu was between 2.2 ng/ml and 25.6 ng/ml (median 10.1 ng/ml). During the first phase of each therapy cycle we observed a decrease to 0.2–3.1 ng/ml (median 1.1 ng/ml).

We also analyzed 14 CSF samples from ALL patients that were taken before the first MTX dose was applied. In all cases an enrichment step was not necessary to determine 5-CH_3-H_4PteGlu because its concentration was high enough for the direct injection method. The levels ranged from 8.0 to 25.8 ng/ml (median 13.1 ng/ml).

4. Discussion

In our laboratory some already existing HPLC methods [12,24–26] were tested. Using the method of Giulidori et al. [26] we could not achieve satisfactory separation of 5-CHO-H_4PteGlu and 5-CH_3-H_4PteGlu. In two cases [12,24] the sensitivity of the 5-CH_3-H_4PteGlu detection (28 and 0.4 ng per injection, respectively) was not sufficient for our purposes. Hahn et al. [25] attained a sensitive determination of 5-CH_3-H_4PteGlu (0.007 ng per injection) using fluorescence detection. For the simultaneous detection of MTX,

Table 3
Recoveries

Compound	Recovery (%)				
	0.1 ng/ml[a]	1 ng/ml[a]	10 ng/ml[a]	100 ng/ml[a]	1000 ng/ml[a]
5-CH_3-H_4PteGlu	94	95	95	96	–[b]
5-CHO-H_4PteGlu	–	–	–	50	47
MTX	–	–	100	103	102
7-OH-MTX	–	–	93	98	94

[a] Concentration level in serum.
[b] – = Not determined.

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Table 4
Concentration range of serum samples taken from children with ALL during treatment

Compound	Concentration range
5-CH_3-H_4PteGlu	0.2–230 ng/ml
5-CHO-H_4PteGlu	0.2–13.7 μg/ml
MTX	0.01–59.4 μg/ml
7-OH-MTX	0.15–54.4 μg/ml

which they used as internal standard, an on-line oxidation procedure was necessary, which however complicated the method. Furthermore, 7-OH-MTX was not analyzed, as is also the case in the paper published by Giulidori et al. [26].

Our method allows the simultaneous determination of MTX, 7-OH-MTX and folinic acid as well as of very low levels of the serum folate 5-CH_3-H_4PteGlu (limit 0.02 ng per injection). This was achieved using HPLC with fluorimetric and ultraviolet detection. In combination with the SPE concentration step, we attained a limit of determination of 0.1 ng/ml for the methyl compound, which is about 1/100 the normal serum concentration in healthy children [7,8].

The lability of the substances and in particular of 5-CH_3-H_4PteGlu is a problem during handling and preparation of the samples. As oxidation of the folates is the main problem, we used L-ascorbic acid as an antioxidant. Several authors used heat treatment [26] or precipitation with perchloric acid [20,24] as deproteinization methods, but we observed high losses of the folates with these procedures, even in the presence of ascorbic acid. Therefore we used SPE to remove proteins, lipids and inorganic ions from the samples. With this mild procedure together with the addition of ascorbic acid to the samples, handling them in the darkened laboratory and thawing them slowly at 4°C, we achieved good stability of the analytes. SPE also results in enrichment of the analytes. The five-fold enrichment we achieved was found to be sufficient for the samples analyzed. However, if necessary a ten-fold concentration effect can be obtained, by subjecting 1 ml of sample to the SPE.

To obtain standardized conditions for the recovery experiments and the calibration curves, we used Serumar, a commercially available serum protein solution. This artificial matrix contains all proteins and ions of human serum [31] but it is folate-free as proved by HPLC analysis. Since recovery experiments with both real serum and serum protein solution showed similar results, Serumar was used in this study. Another advantage is the possibility to calibrate 5-CH_3-H_4PteGlu in the lower concentration range.

With 5-CH_3-H_4PteGlu, MTX and 7-OH-MTX we achieved good recovery values with the SPE procedure at different levels, as can be seen from Table 3. However, 5-CHO-H_4PteGlu showed a poor recovery (about 50%). Because this 5-formyl compound is known to be the most stable folate [6,32], decomposition is an unlikely cause of the high loss. We suspect that on the SPE column, the substance has a broad elution profile, similar to that seen on the HPLC column (see Fig. 2), and a considerable amount passes the SPE column before the final elution step. Experiments with some modifications of the SPE method did not lead to an improvement. Probably a larger amount of SPE material (e.g. 500 mg) or another kind of solid phase, e.g. C_8 or phenyl instead of C_{18}, would give better results. To overcome the problem, we calculated the processed samples using the curve resulting from the analysis of spiked serum protein solutions. Although the recovery of 5-CHO-H_4PteGlu is low we obtained satisfactory linearity of the calibration curve, as indicated by a correlation coefficient of 0.9889. But due to the poor recovery a relatively high limit of determination (100 ng/ml) compared to the other compounds and a high variation during sample preparation (v_{id} = 23% and v_{dd} = 34%) were found.

It has to be emphasized that the described HPLC method does not distinguish between the 6*R*- and 6*S*-isomers of the tetrahydrofolates. Therapeutically, leucovorin is administered as a mixture of the stereoisomers of 5-CHO-H_4PteGlu. However, only the 6*S*-isomer is rapidly converted to [6*S*]-5-CH_3-H_4PteGlu. Therefore, the obtained values for the formyl compound represent mainly the 6*R*-isomer which is of minor interest for clinical purposes because

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it has no biological effect. For therapeutic monitoring, 5-CH_3-H_4PteGlu levels are much more relevant since they reflect the extent of metabolization of the administered [6*S*]-5-CHO-H_4PteGlu. Therefore, the less exact quantification of the formyl folate is acceptable.

In our laboratory the described method was used for pharmacokinetic analysis. One aim of the study was to investigate the influence of MTX therapy on the serum folate content. The measured 5-CH_3-H_4PteGlu levels before start of the therapy (2.2–25.6 ng/ml) are in good agreement with data published for healthy children [7,8]. During the infusion of MTX we observed a decrease to about 10% of the initial level. Even those very low serum concentrations (0.2–3.1 ng/ml) could be reliably measured.

Clinical results of the pharmacokinetic study will be published elsewhere [33].

The method also allowed the analysis of CSF samples. The 14 tested samples were injected directly onto the HPLC column because of their high content. In order to determine low 5-CH_3-H_4PteGlu levels in CSF, the samples can also be subjected to the described sample preparation method.

5. Conclusions

The described method is capable of measuring simultaneously MTX, 7-OH-MTX, 5-CH_3-H_4PteGlu and 5-CHO-H_4PteGlu, substances which are important during high-dose MTX therapy for various malignant diseases. Although there are some problems in determining 5-CHO-H_4PteGlu, the technique is a reliable and valuable tool for therapeutic monitoring and will contribute to more insight in MTX and folate metabolism and to early detection of clinical toxicity.

Acknowledgement

We gratefully thank Dr. Große-Siestrup from the Animal Laboratory of the Klinikum Rudolf Virchow, Berlin for providing us with rabbit liver.

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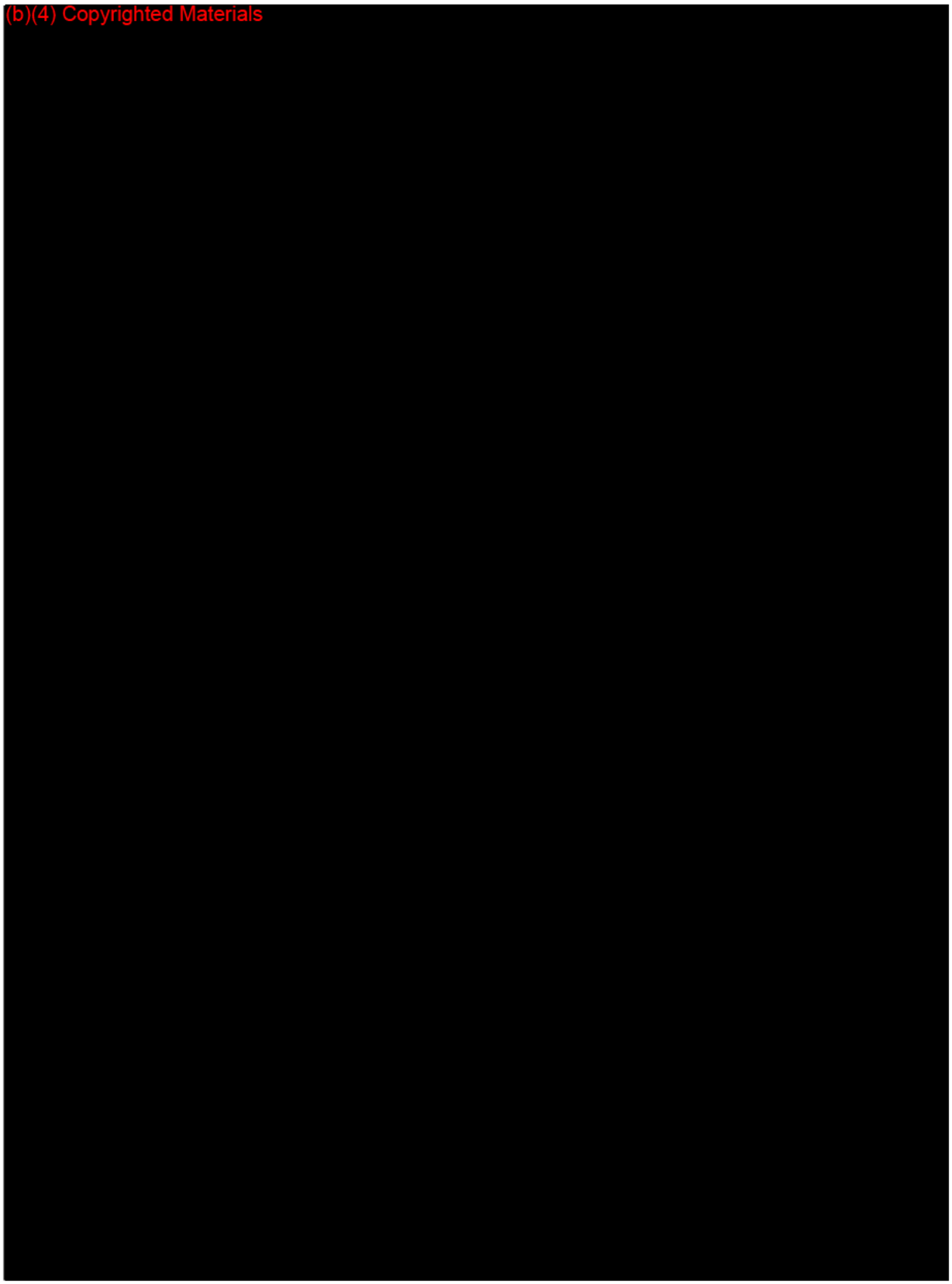

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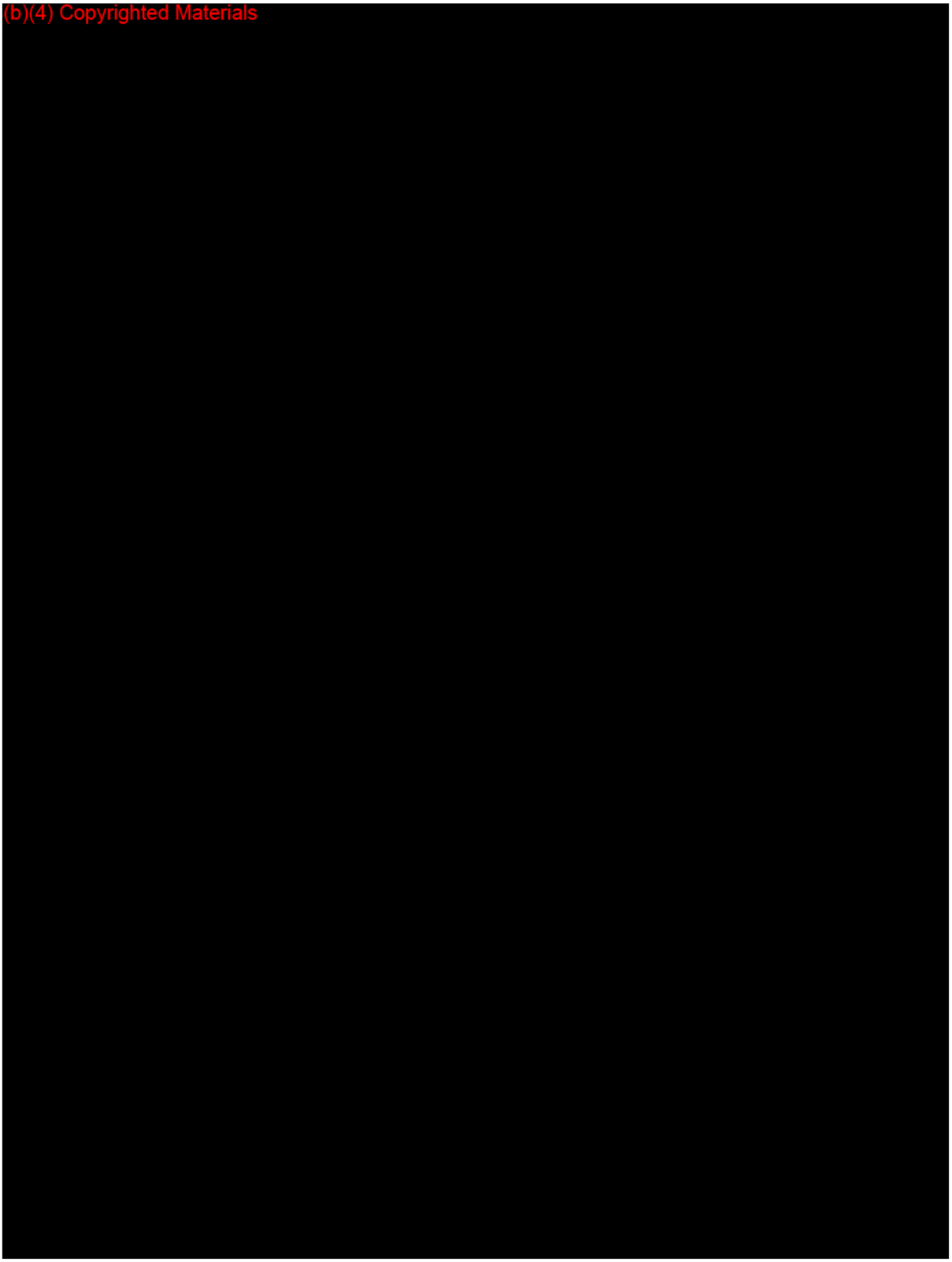

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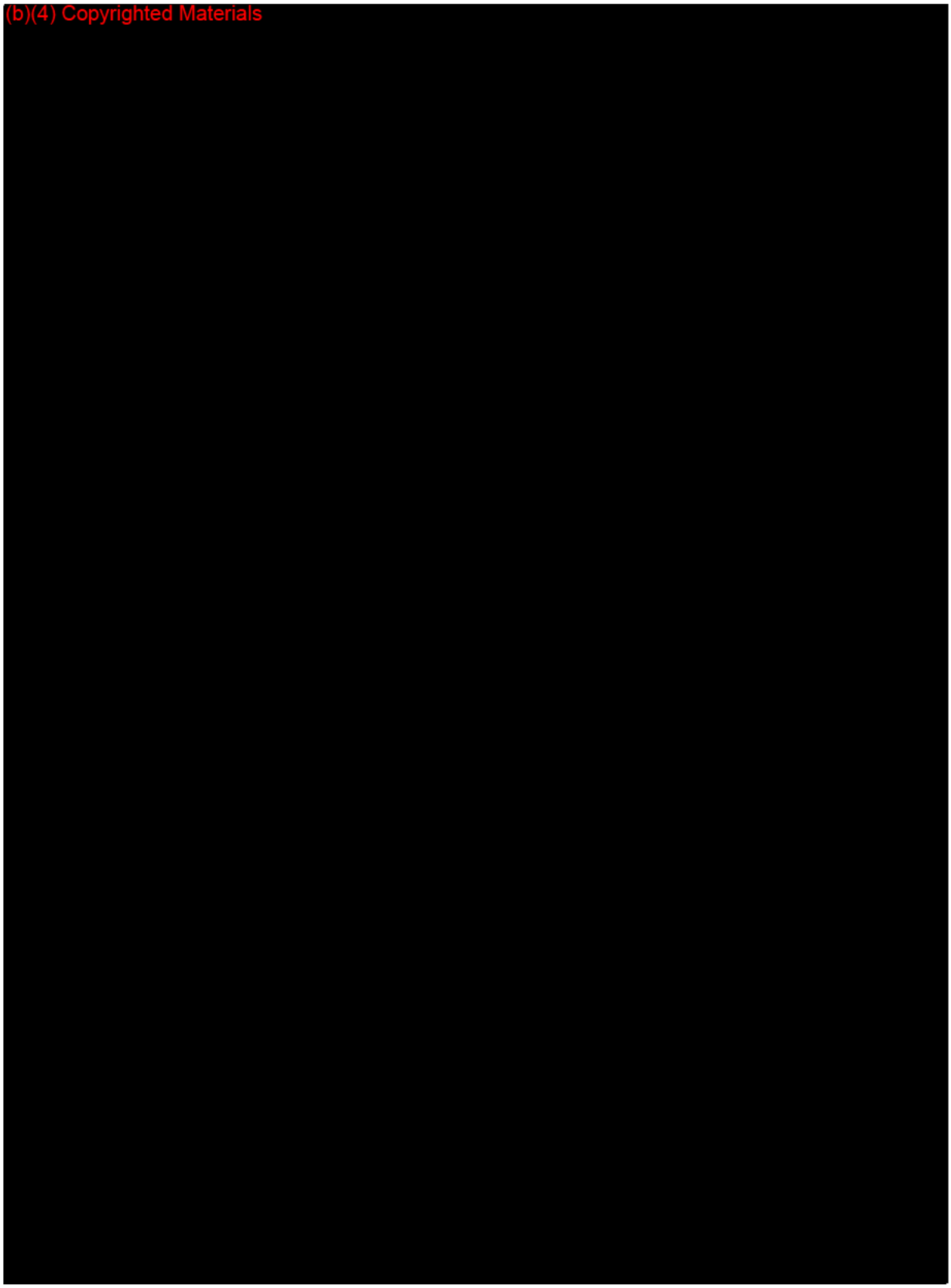

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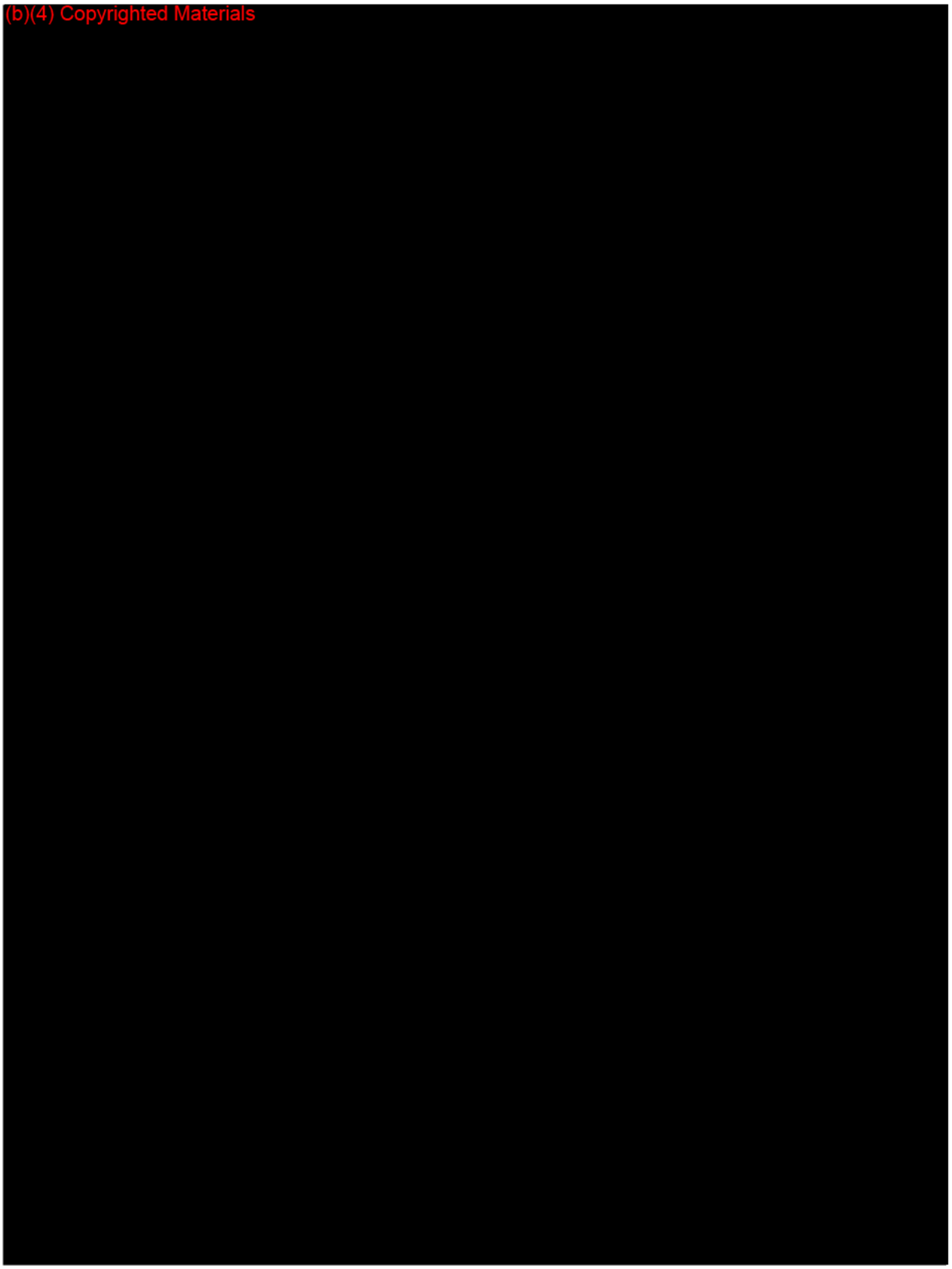

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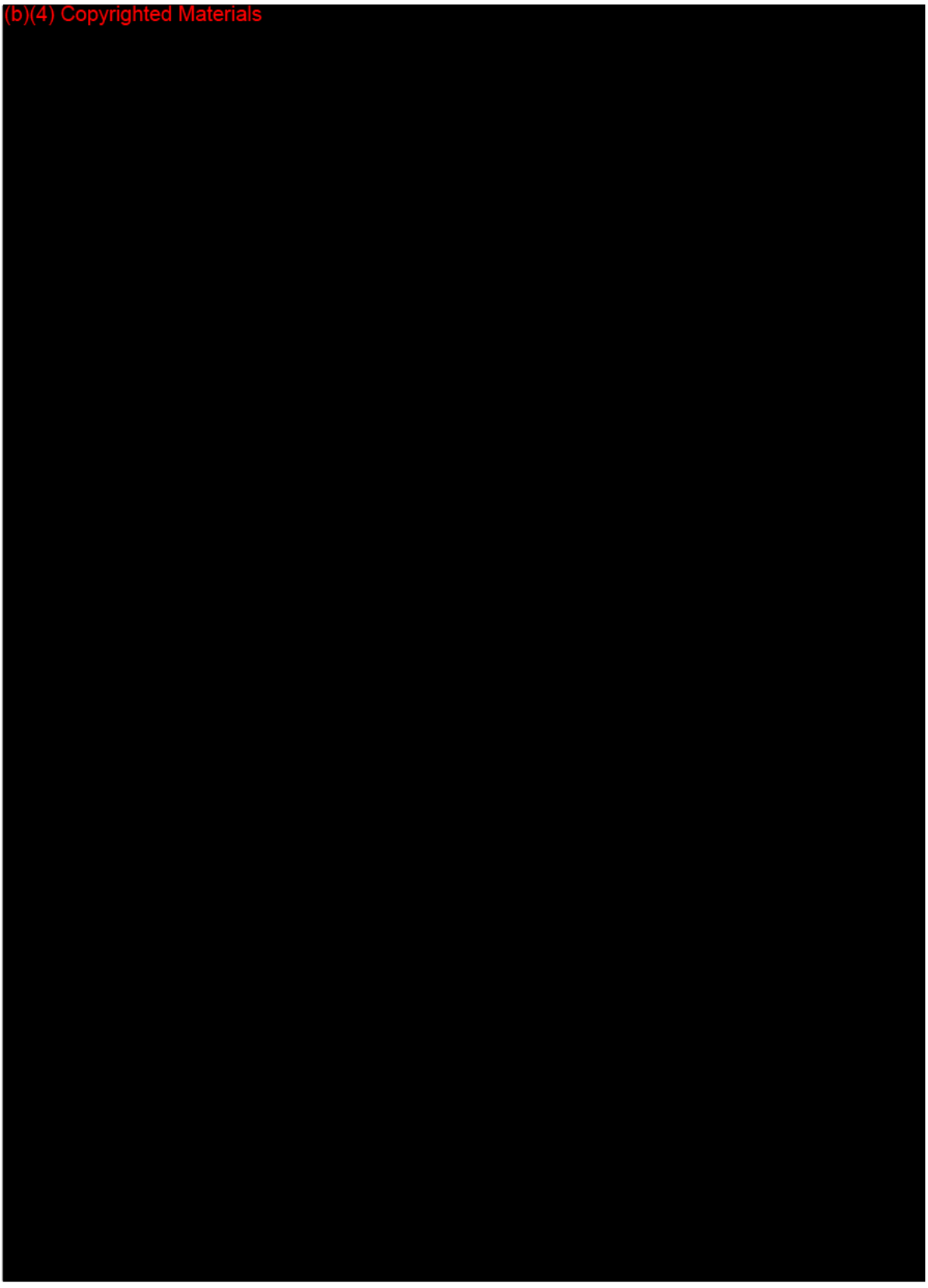

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Clinical case

Annals of Oncology 13: 327–330, 2002
DOI: 10 1093/annonc/mdf021

Significant impairment of high-dose methotrexate clearance following vancomycin administration in the absence of overt renal impairment

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Received 22 February 2001; revised 9 May 2001; accepted 23 May 2001

Background: Methotrexate is an antimetabolite cytotoxic drug which is predominantly renally excreted. Vancomycin, a glycopeptide antibiotic that is used in the febrile neutropaenic patient, can be nephrotoxic. There are no previous reports of any interactions between these two drugs.

Patients and methods: We describe two patients with osteosarcoma treated with high-dose methotrexate-containing chemotherapy who had significantly delayed methotrexate clearance several weeks following exposure to vancomycin.

Results: These patients were treated with alternating chemotherapy consisting of 12 g/m^2 methotrexate, 60 mg/m^2 cisplatin, 75 mg/m^2 adriamycin and 15 g/m^2 ifosfamide. In both patients, serum methotrexate levels fell to below 0.2 μmol/l within 48–96 h during initial treatment cycles. However, following recent exposure to therapeutic vancomycin in the preceding 10 days and in the absence of overt renal impairment, both patients manifested markedly prolonged methotrexate clearance, requiring 170–231 h to reach serum levels of less than 0.2 μM. Subclinical renal impairment was documented by impaired glomerular filtration rates in both cases by technetium 99 m diethylene triamine penta-acetic acid scanning. Subsequent methotrexate cycles using an unmodified schedule were cleared within 72 h. Both cases had their glomerular filtration rate re-assessed, which showed marked improvement.

Conclusions: Recent exposure to vancomycin, even in the absence of overt renal impairment, may adversely affect methotrexate excretion, which can subsequently lead to increased toxicity of the antimetabolite. The glomerular filtration rate should be measured in such cases so that appropriate dose modification of methotrexate can be made.

Key words: delayed excretion, methotrexate, nephrotoxicity, vancomycin

Introduction

Methotrexate is an important drug used in the management of malignancy and connective tissue disorders, such as rheumatoid arthritis. In neoplastic disease, it can be used both in standard doses of 30–40 mg/m^2 for the treatment of breast or bladder cancer, or high doses of 1–12 g/m^2 for lymphoma, leukaemia and osteosarcoma. These higher doses have the potential to cause fatal toxicities and, therefore, methotrexate dose excretion needs to be monitored carefully.

Methotrexate is an inhibitor of dihydrofolate reductase, a critical enzyme in maintaining the intracellular folate pool in its reduced form as tetrahydrofolate, which is important for the synthesis of purine nucleotides and some amino acids [1]. The volume of distribution approaches that of total body water and ~60% is bound to albumin. Methotrexate generally follows a triphasic elimination pattern. The three phases consist of a distribution phase of ~45 min and two elimination phases. Following standard (~40 mg/m^2) or high-dose (500 mg/m^2) methotrexate, the mean elimination half-lives are 2 and 8 h, respectively [2]. The latter two phases are prolonged in patients with renal impairment.

A small proportion of administered methotrexate undergoes hepatic metabolism and is subsequently eliminated by the kidneys. In high doses, 90% of the total methotrexate is renally excreted unchanged. Renal clearance involves glomerular filtration, tubular secretion and tubular resorption. As a result, methotrexate clearance is critically dependent upon renal function [3, 4].

High-dose methotrexate with leucovorin rescue is used in the treatment of osteosarcoma, lymphoma and leukaemia. This treatment is usually given as a prolonged infusion, the duration of which can range from 4 to 48 h. High-dose metho-

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trexate is usually well tolerated provided that patients are adequately hydrated and the urinary pH is kept above 7. Alkalization of the urine increases methotrexate excretion and reduces the risk of precipitation within the renal tubules, which can lead to acute renal failure [5].

The primary toxicities of high-dose methotrexate infusions are myelosuppression and gastrointestinal mucositis. When mucositis occurs, its onset is usually 3–7 days post-treatment and precedes myelosuppression. Myelosuppresssion and mucositis have usually resolved by about day 14. Other toxicities include acute and chronic hepatitis, self-limiting pneumonitis and central nervous system disturbances [1]. The risk of potentially fatal toxicity is significantly increased with high-dose methotrexate in the setting of renal impairment and is related to prolonged periods of exposure due to elevated serum levels. At 24 and 48 h, toxicity is greater if the serum levels are above 20 and 2 μmol/l, respectively [6].

Vancomycin is a glycopeptide antibiotic which inhibits cell wall synthesis. It is active against Gram-positive bacteria, including methicillin-resistant staphylococci. It is widely distributed in total body water and has a plasma half-life of 6 h. Elimination is virtually entirely by glomerular filtration. Side effects include infusional reactions, which may be anaphylactoid or the 'redman syndrome', and auditory and vestibular problems including impaired hearing, tinnitus and, occasionally, vestibular dysfunction [7]. Other side effects include renal impairment manifested by increasing urea and creatinine, especially in patients exposed to high trough serum vancomycin concentrations [8]. This is more likely to occur when patients are concomitantly exposed to aminoglycosides or in the presence of pre-existing renal impairment [9]. This drug-related azotaemia usually resolves within days of drug cessation.

We report two patients with osteosarcoma treated with a chemotherapy regime containing repeated high-dose methotrexate in whom, initially, there was normal clearance of methotrexate and no toxicity. However, when exposed to vancomycin within the preceding 10 days, they had prolonged methotrexate excretion and subsequent toxicity.

Case 1

A 29-year-old man was referred to our institute for management of distal femoral osteosarcoma. He received preoperative chemotherapy consisting of repeated cycles of 12 g/m^2 methotrexate, 60 mg/m^2 cisplatin, 75 mg/m^2 adriamycin and 15 g/m^2 ifosfamide [2, 9]. The patient had no significant past medical history predisposing him to renal impairment and took no regular medications. The initial physical examination, which included blood pressure and urine analysis, was normal. Initial serum levels of creatinine and urea were 0.1 mmol/l (normal range: 0.06–0.13 mmol/l) and 7 mmol/l (2.5–7.7 mmol/l), respectively. During day 1 of treatment he received 23 g methotrexate (12 g/m^2) intravenously (i.v.) over 4 h.

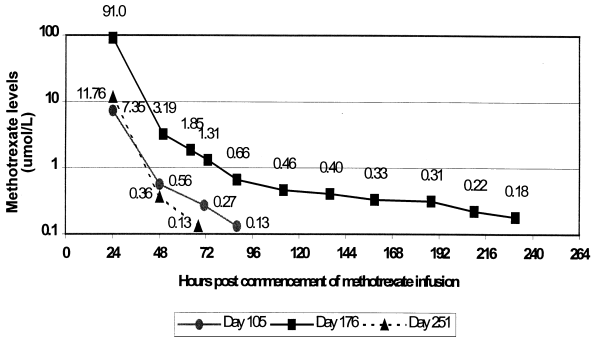

Figure 1. Fall of serum methotrexate concentration over time, case 1.

Twenty hours post completion of methotrexate treatment, he commenced folinic acid i.v. every 6 h, receiving a total of 18 doses. Urine pH was kept above 7.0 during methotrexate elimination. The serum methotrexate level fell to a safe level of 0.2 μmol/l within 72 h (Figure 1). There were no major toxicities from this or subsequent chemotherapy courses. During day 49 he received a second identical course of 23 g methotrexate (12 g/m^2) without complication.

He was admitted during day 130 with a non-neutropaenic febrile illness, following recently administered cisplatin. Serum levels of creatinine and urea were 0.07 mmol/l and 3.7 mmol/l, respectively. Vancomycin (1 g) twice daily, 1 g cefepime three times daily and 240 mg gentamicin daily were commenced on the first day of admission. A vancomycin trough level on day 131, following the third dose, was 5 mmol/l (<10 mmol/l) but was found to be 59 mmol/l after the sixth dose. Subsequent vancomycin doses were withheld. Gentamicin levels were closely monitored and always fell within the therapeutic range. *Acinetobacter calcoaceticus* and *Strenotrophomonas maltophilia* were isolated, the peripheral access was removed and oral bacterium was given for 1 week. During the entire admission the patient's renal function remained normal.

He was re-admitted on day 168 with a febrile neutropaenic episode post high-dose ifosfamide. Again he was commenced on 1 g vancomycin twice daily and 1 g cefepime i.v. three times daily. A vancomycin trough level after three doses was 5 mmol/l. Vancomycin was ceased after five doses. No source of infection was identified. His renal function remained normal throughout the admission with creatinine and urea levels in the range of 0.08–0.09 mmol/l and 5.9–6.9 mmol/l, respectively. A lower total dose of 20.2 g (12 g/m^2) methotrexate was administered on day 176 because of a reduced body surface area (BSA). At the time, the serum creatinine was 0.08 mmol/l and the urea was 6.9 mmol/l. Folinic rescue was administered in an identical manner to previous cycles. Methotrexate levels remained above 0.2 μmol/l for 231 h (Figure 1). During that time urine was well alkalinized with the pH maintained above 7. No other renally toxic agents were administered during the admission. Serum creatinine ranged from 0.08 to 0.16 mmol/l over this time. The patient developed grade III mucositis.

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This had not been a feature of previous high-dose methotrexate cycles. On day 183 a quantitative glomerular filtration rate (GFR) scan using technetium 99 m diethylene triamine penta-acetic acid (DTPA) was performed measuring a value of 64 ml/min. The subsequent methotrexate dose was administered on day 251 and the serum level fell to less than 0.2 μM within 96 h. A further renal scan was performed during day 260 revealing a GFR of 100 ml/min, and the final scheduled methotrexate dose of 20.2 g (12 g/m^2) was cleared within 3 days (Figure 1).

Case 2

A 29-year-old woman was admitted for chemotherapy for distal left femur osteosarcoma prior to and following limb-sparing surgery according to the previously mentioned protocol. She had no significant past medical history predisposing her to renal impairment. The only regular medication prior to commencement of treatment was the oral contraceptive pill. During her first admission for chemotherapy, her clinical examination, which included blood pressure and urine analysis, was normal. Her renal function was normal with creatinine and urea levels of 0.08 mmol/l and 3.8 mmol/l, respectively. On day 1 the dose of methotrexate given was 20 g (12 g/m^2) followed by folinic rescue as previously described. Methotrexate fell to a safe level below 0.2 μmol/l within 72 h (Figure 2). Subsequent chemotherapy was given without major complications, including the day 49 methotrexate dose of 20 g (12 g/m^2). On day 224 the patient was admitted with methicillin-resistant staphylococcus epidermidis septicaemia complicating pancytopaenia following recently administered ifosfamide. On admission her creatinine and urea were 0.07 and 3.7 mmol/l, respectively. Cefepime (1 g) i.v. three times daily was immediately commenced and 1 g vancomycin twice daily was added on day 2 due to persisting fevers. A vancomycin trough level taken after the third dose was 4 mg/l. A repeat trough level after the sixth dose was 12.5 mg/l, following which the vancomycin dose was reduced to 750 mg i.v. twice daily. It was ceased after a total of 10 doses. Her serum creatinine and urea remained within the normal range: 0.07–0.09 and 3.2–3.5 mmol/l, respectively. On day 233 she received a methotrexate dose of 20 g (12 g/m^2) with a schedule identical to the previous doses. Her serum creatinine and urea were 0.09 mmol/l and 3.5 mmol/l, respectively. Folinic rescue was given in an identical manner to previous cycles. The patient was well hydrated and urine pH did not drop below 7. It took 170 h for the serum methotrexate level to fall below 0.2 μmol/l (Figure 2). The patient suffered grade 2 mucositis, which she had not had with previous methotrexate treatments. No concomitant nephrotoxic agents were administered. A renal scan performed on day 241 revealed bilaterally impaired renal function with a GFR value of 87 ml/min. Repeat GRF prior performed on day 317 estimated the GFR to be 100 ml/min. Her last methotrexate dose was administered on day 328. She was given a lower methotrexate dose of 19 g (12 g/m^2) because of a reduced BSA. Her renal function according to serum creatinine and urea were normal. She again cleared the drug under 72 h (Figure 2).

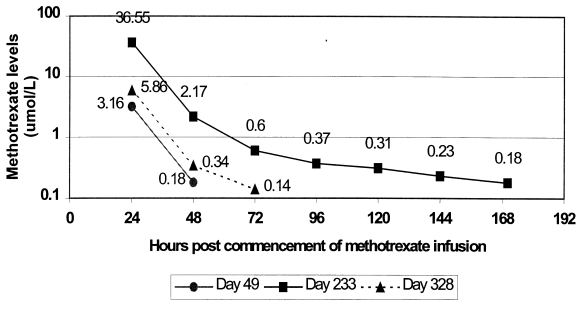

Figure 2. Fall of serum methotrexate concentration over time, case 2.

Discussion

We describe two patients with osteosarcomas, treated with chemotherapy containing high-dose methotrexate, who manifest impaired methotrexate excretion soon after exposure to vancomycin. Both patients had previously received several cycles of high-dose methotrexate with prompt excretion of the drug. Although the chemotherapy regime contained several potentially nephrotoxic agents, including methotrexate, ifosfamide and cisplatin, it was only after recent exposure to vancomycin that methotrexate excretion was significantly delayed. While it is possible that the concomitant administration of other medications may have altered the extent of protein binding of methotrexate, one would predict that this would primarily affect the peak plasma concentration rather than drug clearance [10]. Not only did both patients have higher peak serum levels following recent exposure to vancomycin, but the excretion was markedly delayed, which could not be explained by displacement from protein binding alone. Our proposed hypothesis is that vancomycin can cause subclinical nephrotoxicity, which may interfere with the excretion of methotrexate.

The mechanism of vancomycin-induced nephrotoxicity is not well understood. In animal studies, vancomycin can cause dilation of the lumina of the renal tubules, dilation of the Bowman's capsule, destruction of renal epithelial cells and formation of hyaline casts [7]. Electron microscopic examination revealed an increase in the number of vacuoles and lysosymes, swollen mitochondria and deformed nuclei of the tubular epithelia [11]. This would suggest that the major site of damage is the renal tubules. Such damage would be predicted to interfere with methotrexate excretion, as renal handling of the drug is predominantly dependent on tubular function. In both cases, there was documented impaired glomerular filtration measured by a quantitative GRF scan using technetium 99 m DTPA. This test measures GFR and not tubular function. However, if there is sufficient tubular

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damage resulting in desquamated epithelia blocking the tubules, impaired drainage of the nephron could result in an impaired GFR measurement. One problem is that the GFRs were not measured before the prolonged methotrexate exposure. Therefore, it is difficult to be certain whether the specific degree of renal impairment measured was wholly attributable to previous vancomycin exposure. Some of the subsequent impairment of GRF may have been due to the prolonged elevated methotrexate serum levels. However, the nephrotoxic effects of methotrexate are also predominantly tubular.

Because methotrexate is predominantly renally excreted, any nephrotoxicity resulting from recent exposure to vancomycin would potentiate the side effect profile of the antimetabolite. This is particularly important in the case of high-dose methotrexate, where the toxicities are potentially fatal. Renal impairment may occur in the face of normal serum creatinine and urea (noted at the time of methotrexate administration in both cases) and non-toxic trough levels of vancomycin (illustrated by the second case only). Such subclinical nephrotoxicity can be assessed by measuring GFR with a DTPA renal scan. In this way, dose modification of methotrexate can be made according to the GFR measured, to minimize any significant complications of treatment.

In conclusion, the administration of vancomycin, an antibiotic commonly used in cancer patients who have treatment-induced febrile neutropaenia, can result in renal impairment. This nephrotoxicity may not be detected by the measurement of serum urea and creatinine concentrations. When methotrexate has been administered in the proceeding 7–10 days, excretion of the drug can be impaired, resulting in a prolonged systemic exposure to the cytotoxic drug. This results in potentially significant toxicity, particularly in the case of high doses, where the outcome may be fatal. To minimize such complications, we believe it would be prudent to assess GFR in this situation, to allow dose modification of methotrexate if necessary.

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CANCER DRUG DELIVERY
Volume 4, Number 3, 1987
Mary Ann Liebert, Inc., Publishers

Pharmacokinetics of Methotrexate and 7-Hydroxy-Methotrexate After Methotrexate Infusions

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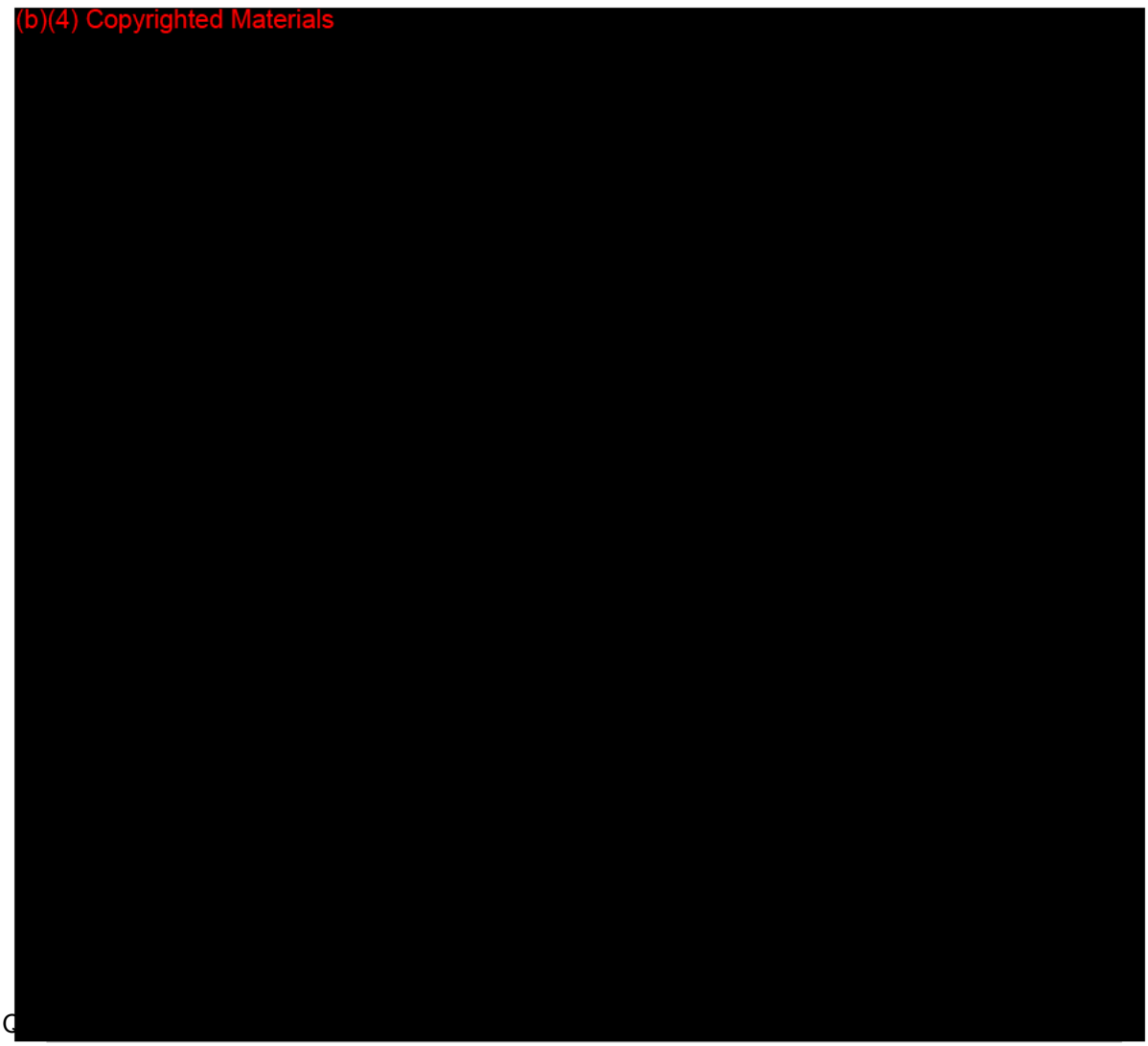

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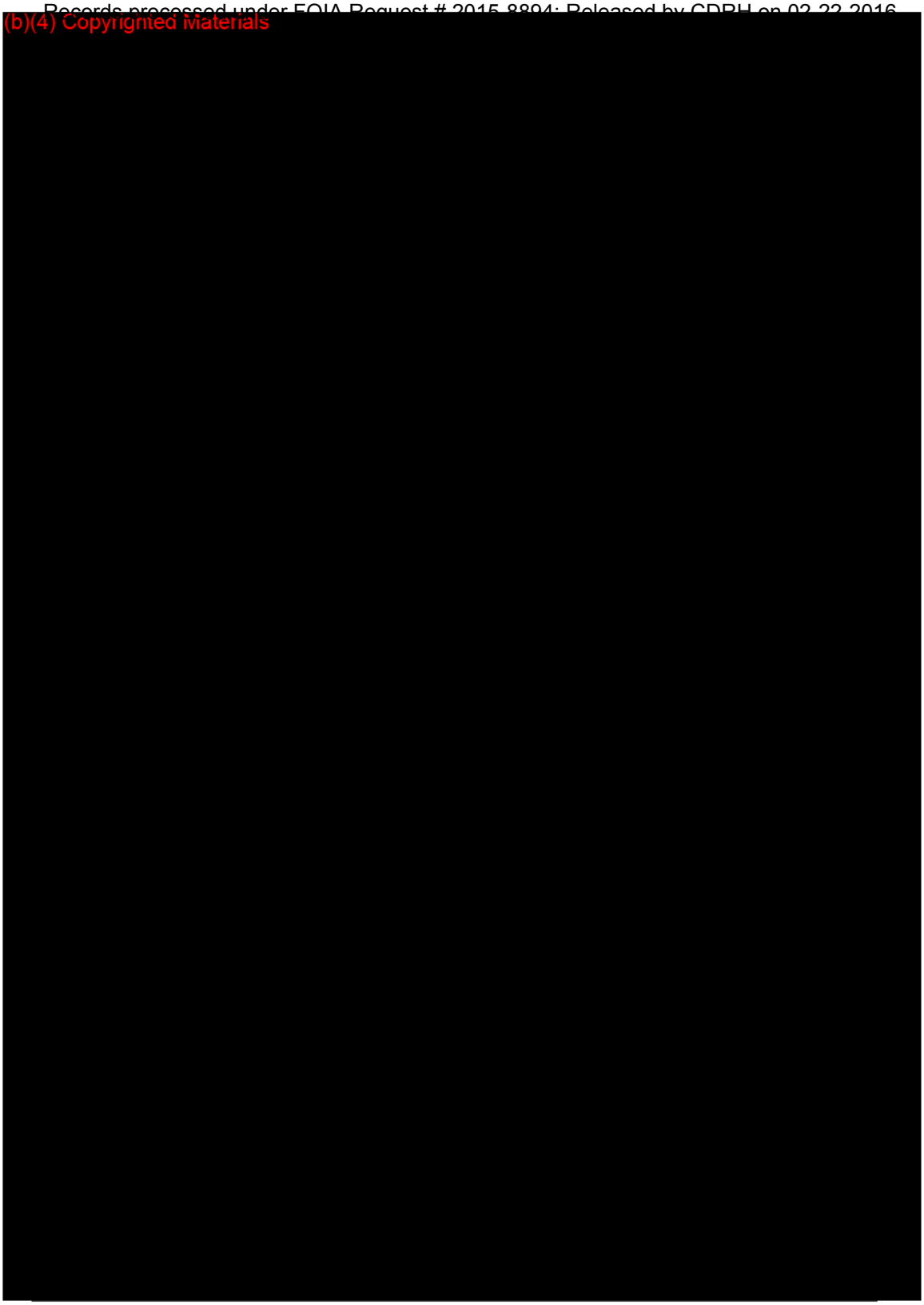

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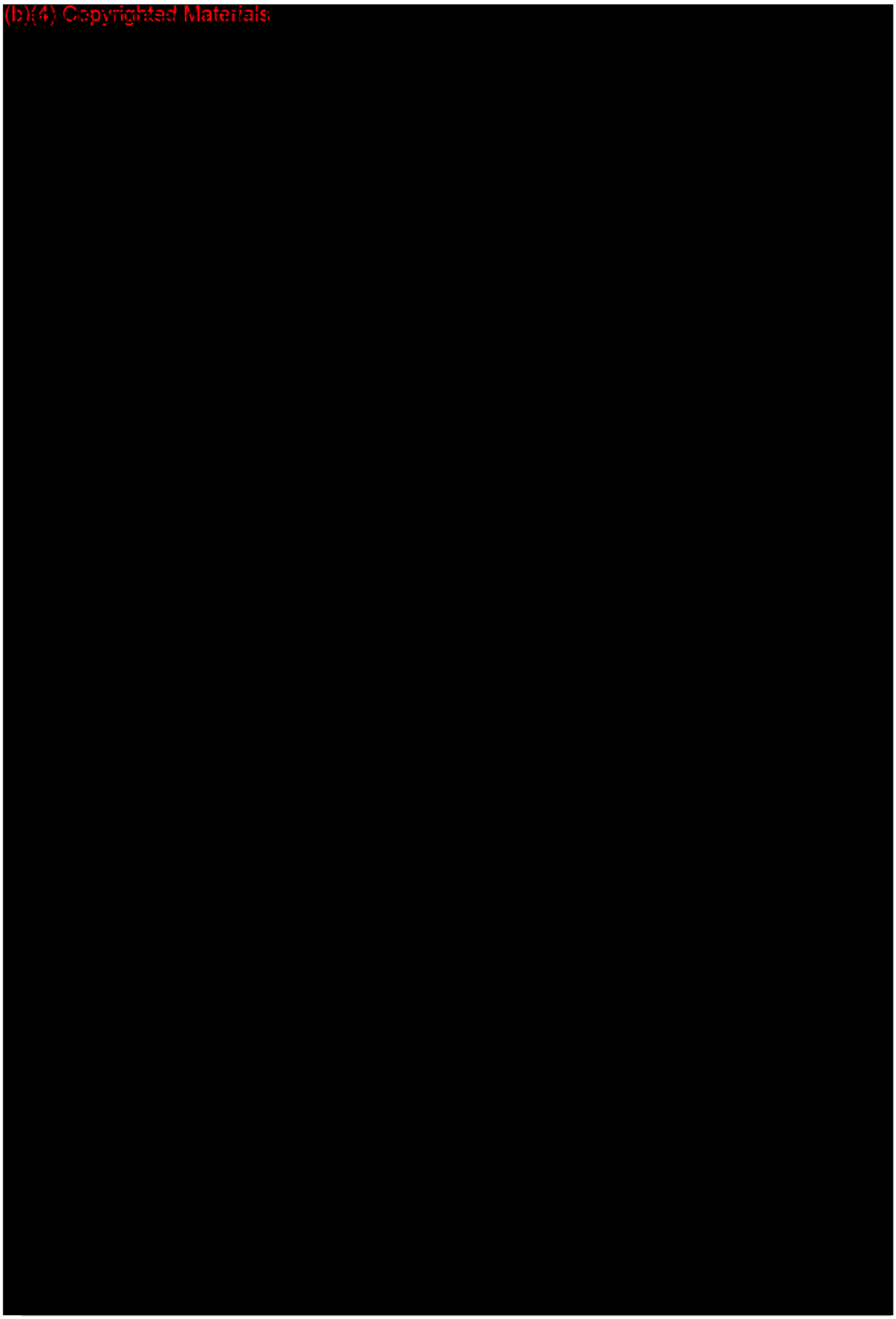

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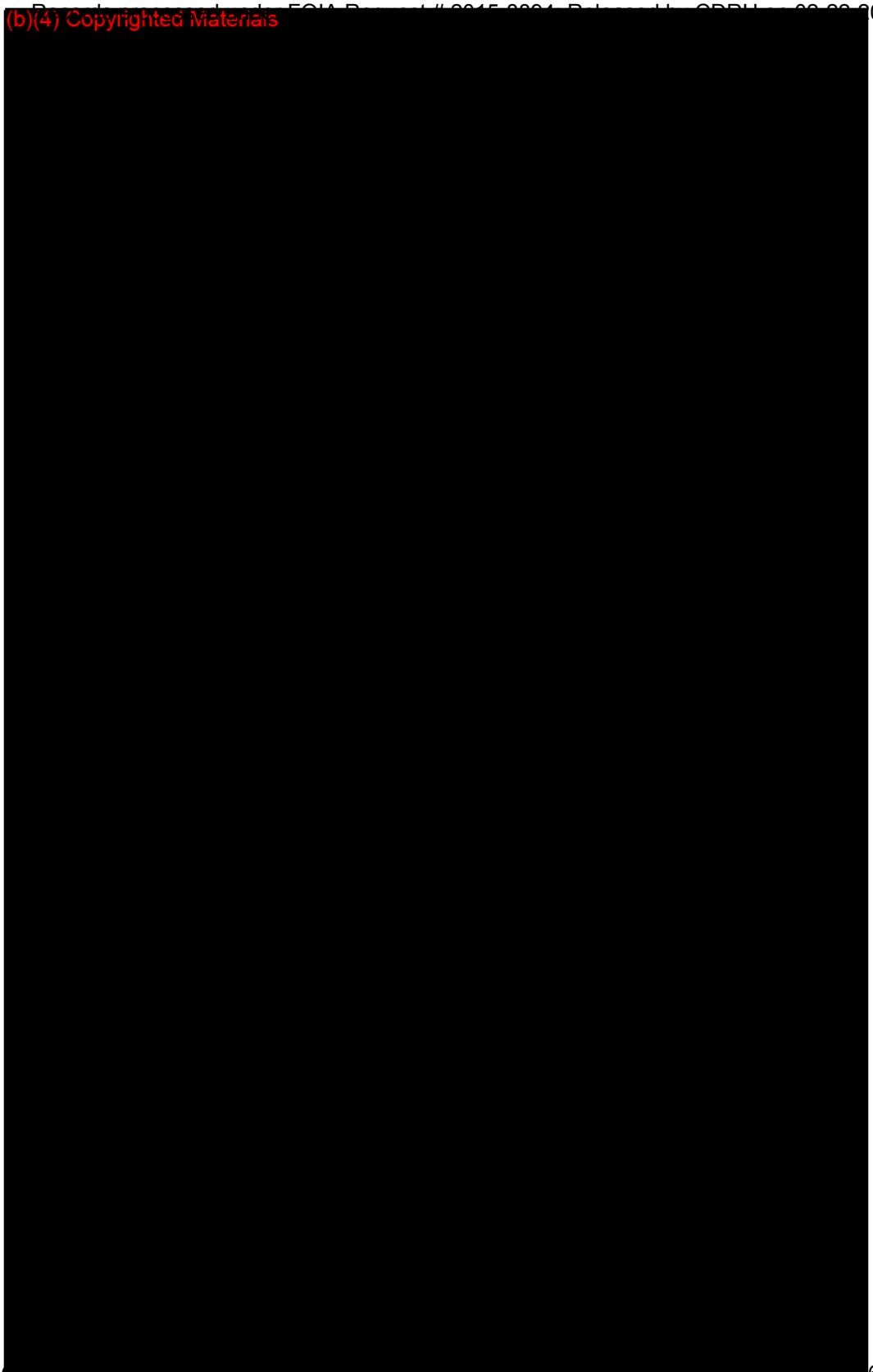

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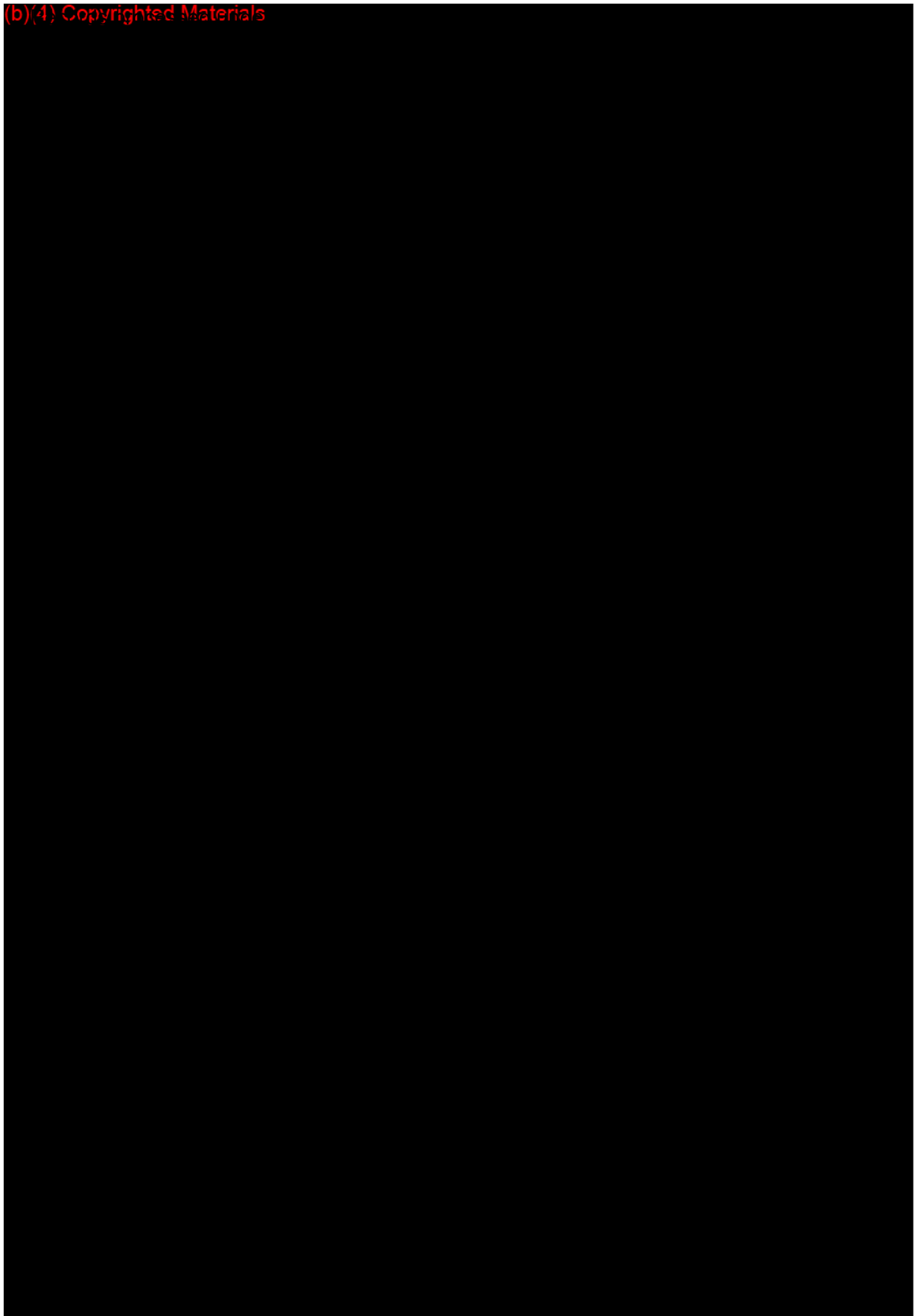

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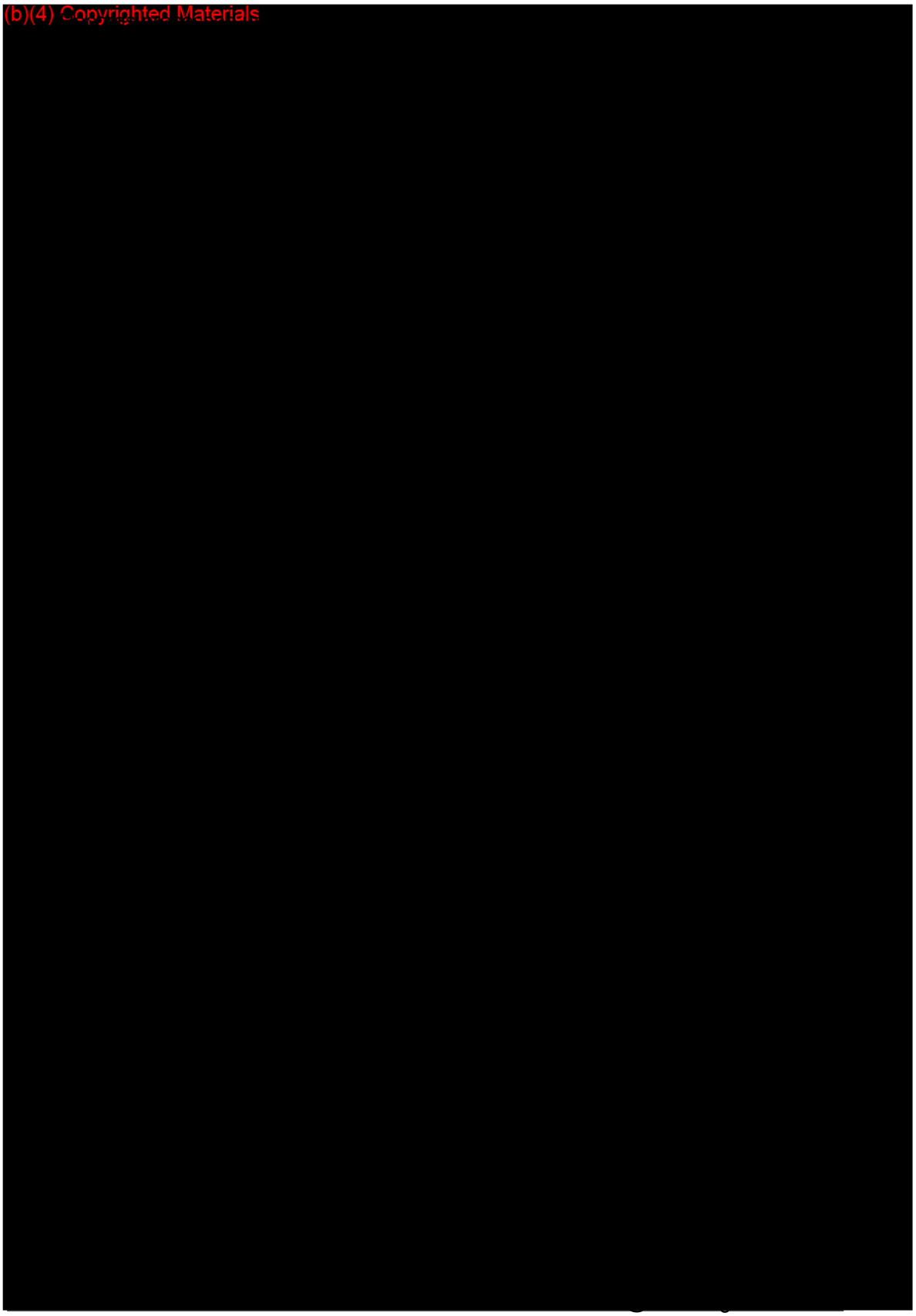

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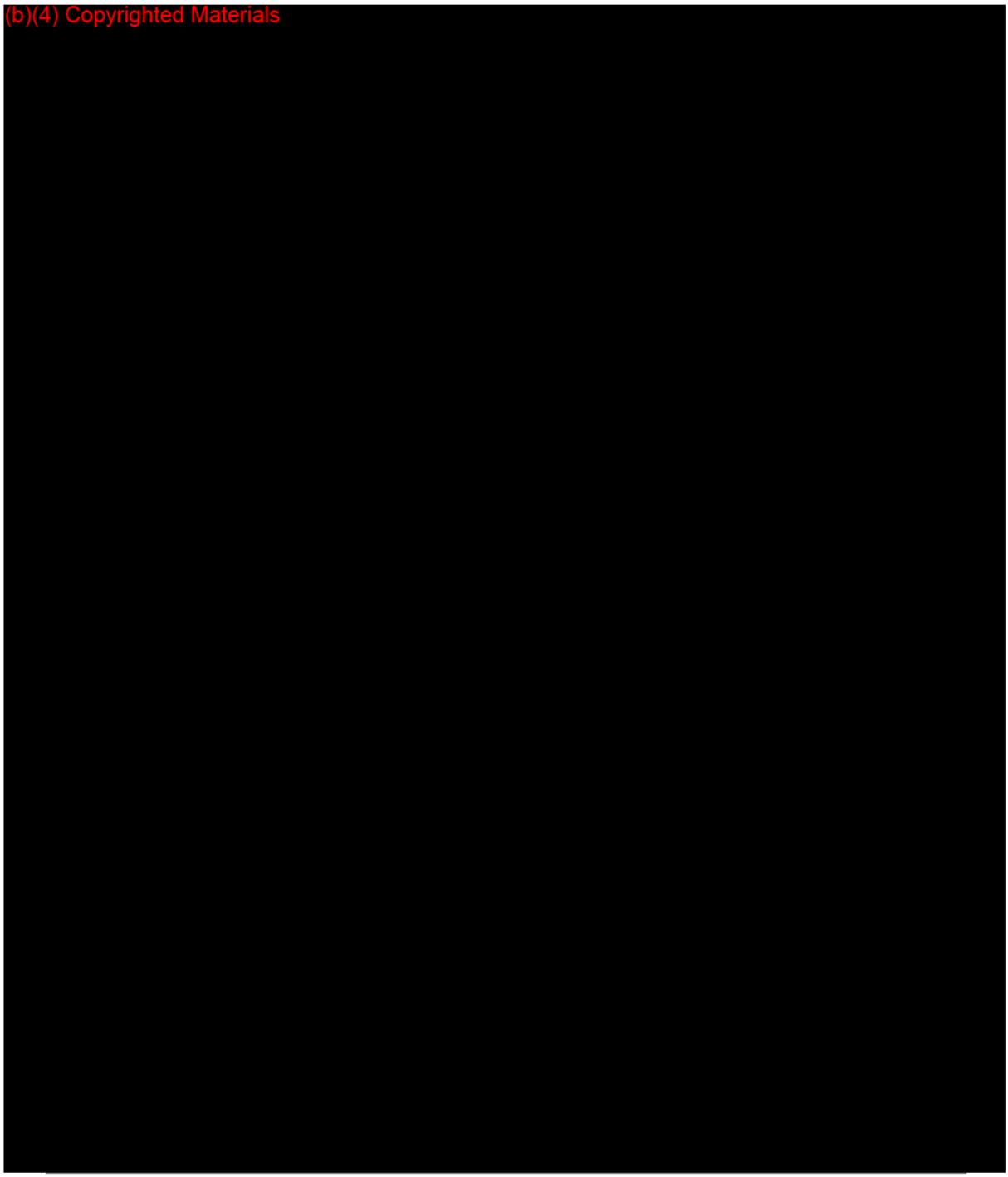

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Pharmacokinetics of Methotrexate and 7-Hydroxymethotrexate Following Infusions of High-Dose Methotrexate[1,2]

Henning Breithaupt[3,*] and Eberhard Küenzlen[4,5]

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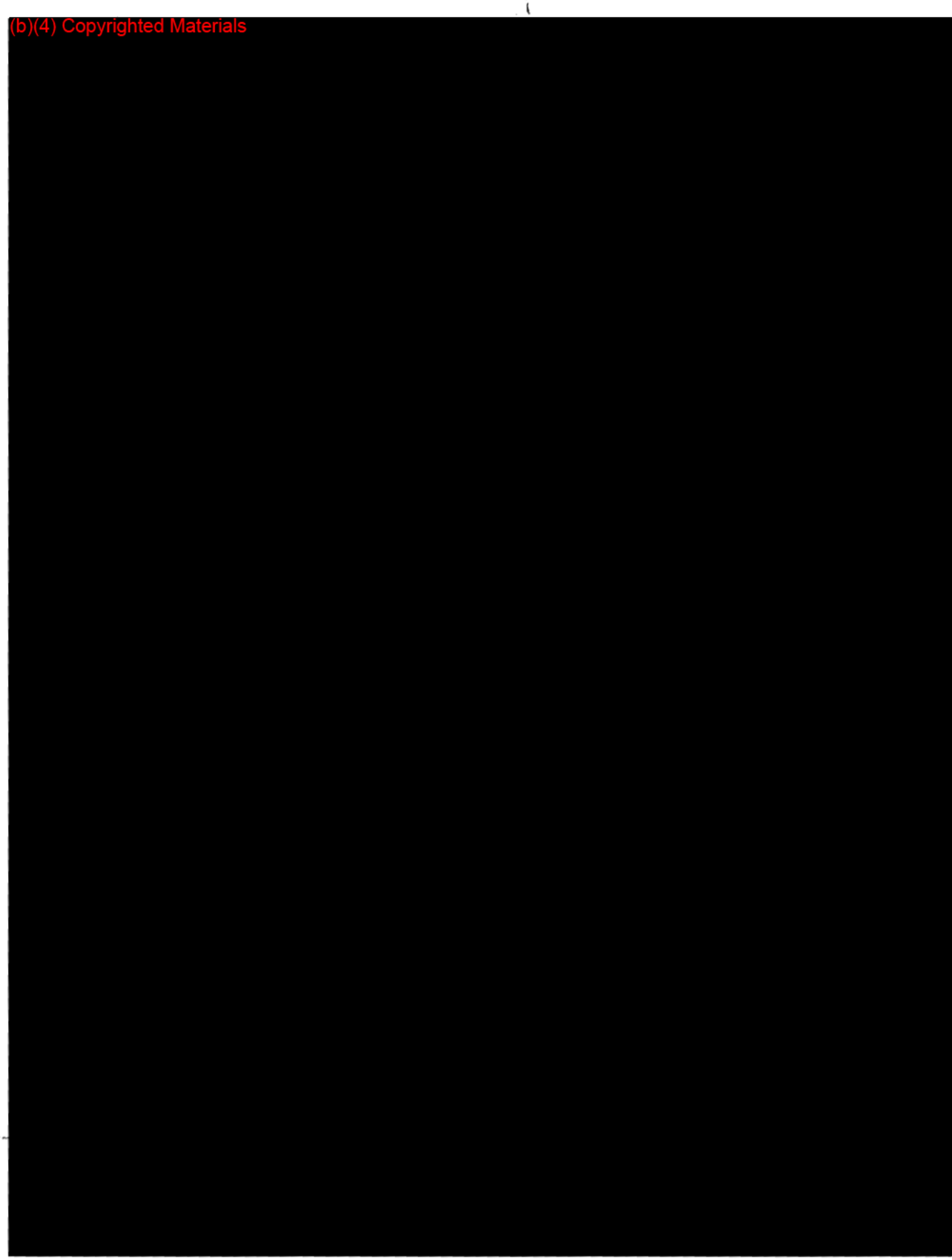

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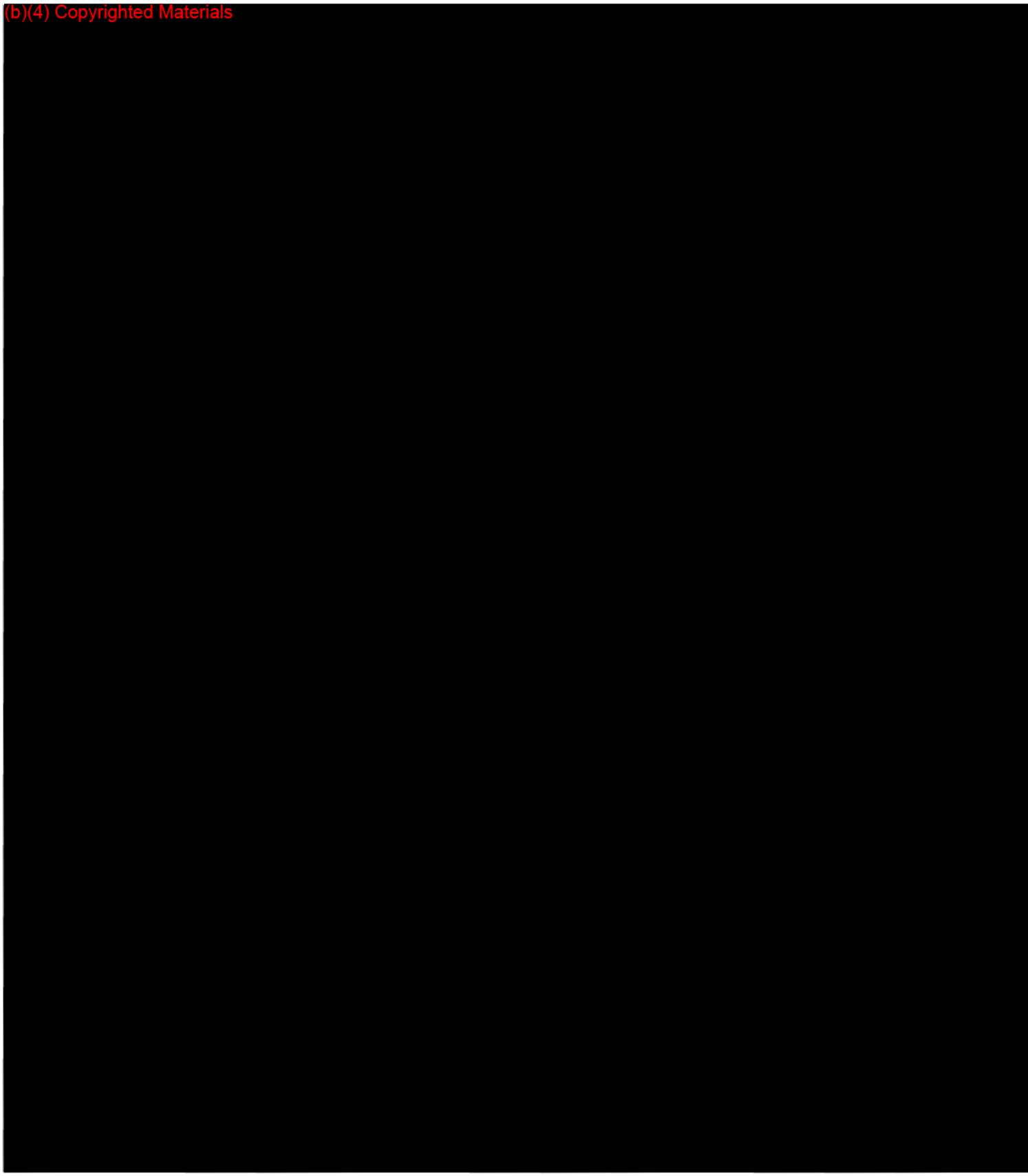

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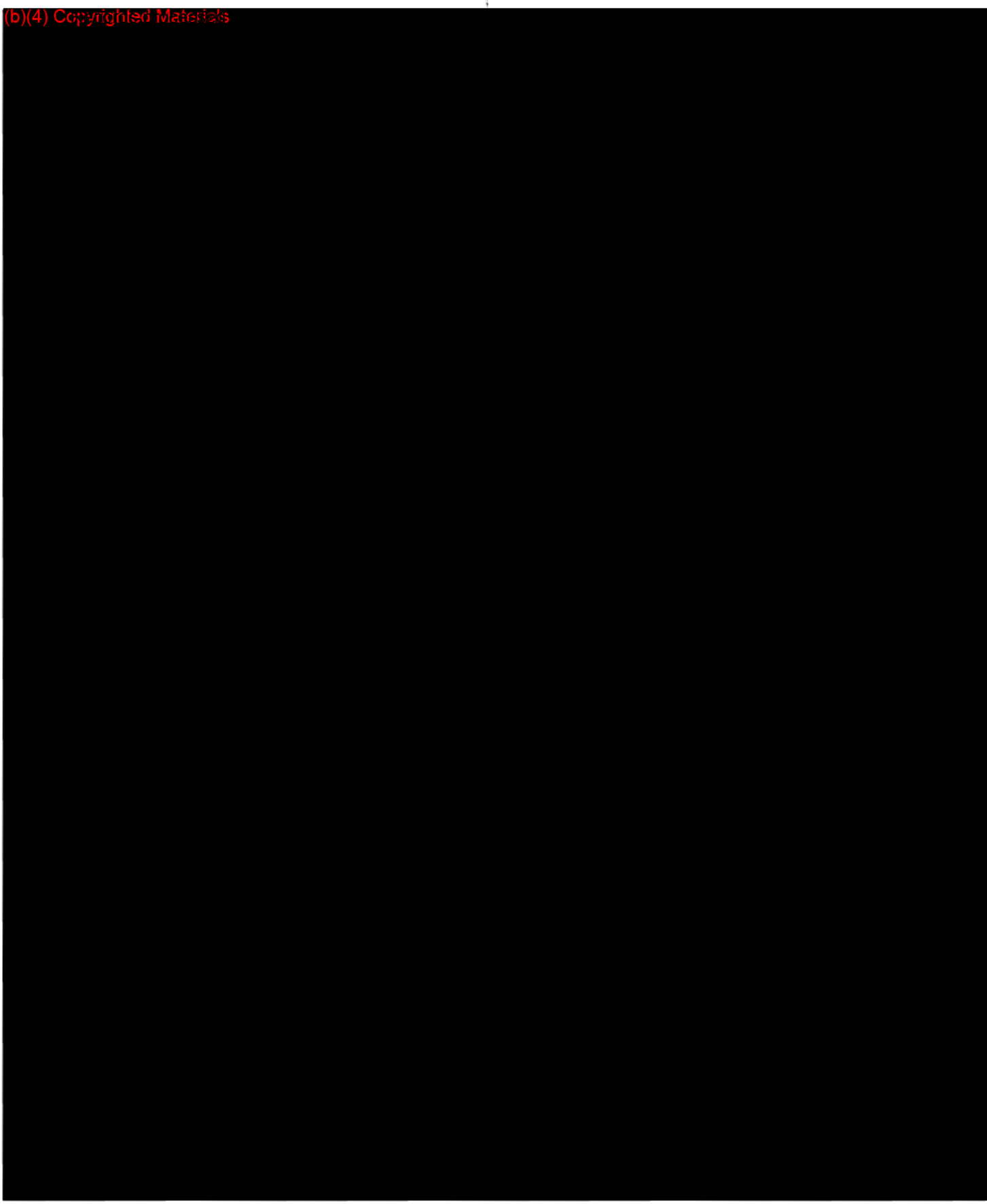

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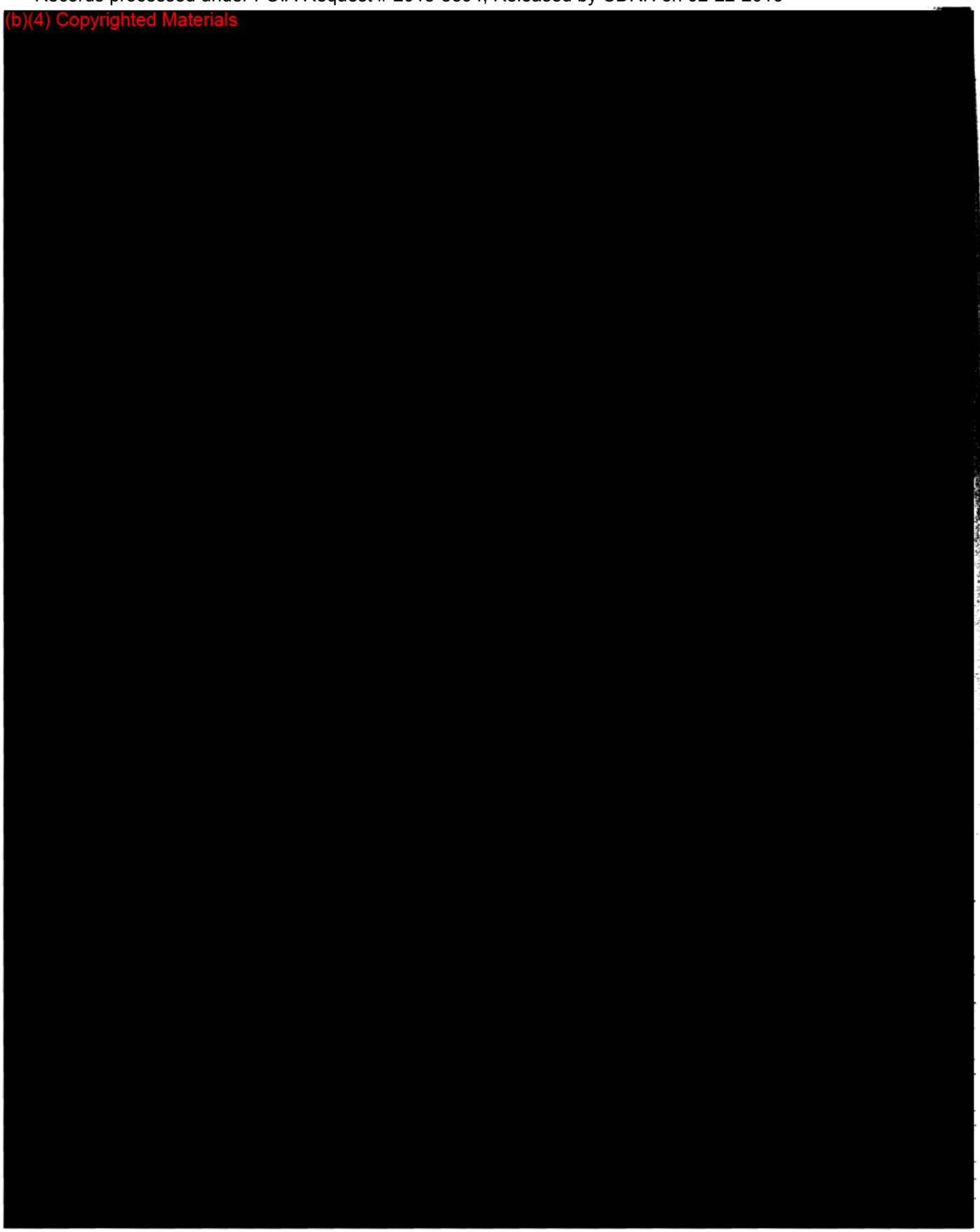

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British Journal of Cancer (2005) 92, 480–487
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www.bjcancer.com

Carboxypeptidase G_2 rescue in patients with methotrexate intoxication and renal failure

S Buchen[1], D Ngampolo[1], RG Melton[2], C Hasan[1], A Zoubek[3], G Henze[4], U Bode[1] and G Fleischhack*[,1]

[1]Department of Paediatric Haematology/Oncology, Children's Medical Hospital, University of Bonn, Bonn, Germany; [2]Protherics Plc, Salisbury, UK; [3]St Anna Children's Hospital, Vienna, Austria; [4]Department of Paediatric Haematology/Oncology, Charité, Humboldt University of Berlin, Germany

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J M Chessells, T C Cox, B Kendall, et al.

Arch Dis Child 1990 65: 416-422
doi: 10.1136/adc.65.4.416

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank (42 U.S.C. § 282(j))

(For submission with an application/submission, including amendments, supplements, and resubmissions, under §§ 505, 515, 520(m), or 510(k) of the Federal Food, Drug, and Cosmetic Act or § 351 of the Public Health Service Act.)

SPONSOR / APPLICANT / SUBMITTER INFORMATION

1. NAME OF SPONSOR/APPLICANT/SUBMITTER
   ARK Diagnostics Inc.

2. DATE OF THE APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES
   Jul 1, 2011

3. ADDRESS *(Number, Street, State, and ZIP Code)*
   1190 Bordeaux Drive
   Sunnyvale, CA 94089

4. TELEPHONE AND FAX NUMBERS *(Include Area Code)*
   (Tel.) 408-747-0700
   (Fax) 408-747-0783

PRODUCT INFORMATION

5. **FOR DRUGS/BIOLOGICS:** Include Any/All Available Established, Proprietary and/or Chemical/Biochemical/Blood/Cellular/Gene Therapy Product Name(s)
   FOR DEVICES: Include Any/All Common or Usual Name(s), Classification, Trade or Proprietary or Model Name(s) and/or Model Number(s)
   (Attach extra pages as necessary)

ARK Methotrexate Assay, 5026-0001-00

ARK Methotrexate Calibrator, 5026-0002-00

ARK Methotrexate Control, 5026-0003-00 (complete set)

ARK Methotrexate Dilution Buffer 5026-0004-00

ARK Methotrexate Control, 5026-0003-01 (calibration range subset)

ARK Methotrexate Control, 5026-0003-02 (high range subset)

APPLICATION / SUBMISSION INFORMATION

6. TYPE OF APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

☐ IND ☐ NDA ☐ ANDA ☐ BLA ☐ PMA ☐ HDE ☒ 510(k) ☐ PDP ☐ Other

7. INCLUDE IND/NDA/ANDA/BLA/PMA/HDE/510(k)/PDP/OTHER NUMBER *(If number previously assigned)*

8. SERIAL NUMBER ASSIGNED TO APPLICATION/SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES

CERTIFICATION STATEMENT / INFORMATION

9. CHECK ONLY ONE OF THE FOLLOWING BOXES *(See instructions for additional information and explanation)*

☒ A. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply because the application/submission which this certification accompanies does not reference any clinical trial.

☐ B. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, do not apply to any clinical trial referenced in the application/submission which this certification accompanies.

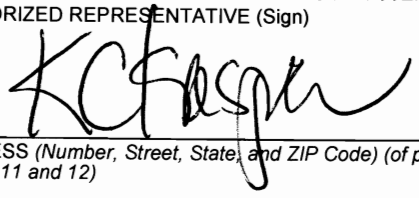
☐ C. I certify that the requirements of 42 U.S.C. § 282(j), Section 402(j) of the Public Health Service Act, enacted by 121 Stat. 823, Public Law 110-85, apply to one or more of the clinical trials referenced in the application/submission which this certification accompanies and that those requirements have been met.

10. IF YOU CHECKED BOX C, IN NUMBER 9, PROVIDE THE NATIONAL CLINICAL TRIAL (NCT) NUMBER(S) FOR ANY "APPLICABLE CLINICAL TRIAL(S)," UNDER 42 U.S.C. § 282(j)(1)(A)(i), SECTION 402(j)(1)(A)(i) OF THE PUBLIC HEALTH SERVICE ACT, REFERENCED IN THE APPLICATION/ SUBMISSION WHICH THIS CERTIFICATION ACCOMPANIES *(Attach extra pages as necessary)*

NCT Number(s):

The undersigned declares, to the best of her/his knowledge, that this is an accurate, true, and complete submission of information. I understand that the failure to submit the certification required by 42 U.S.C. § 282(j)(5)(B), section 402(j)(5)(B) of the Public Health Service Act, and the knowing submission of a false certification under such section are prohibited acts under 21 U.S.C. § 331, section 301 of the Federal Food, Drug, and Cosmetic Act. **Warning:** A willfully and knowingly false statement is a criminal offense, U.S. Code, title 18, section 1001.

11. SIGNATURE OF SPONSOR/APPLICANT/SUBMITTER OR AN AUTHORIZED REPRESENTATIVE (Sign)



12. NAME AND TITLE OF THE PERSON WHO SIGNED IN NO. 11
   (Name) Kenneth C. Kasper, PhD
   (Title) Executive Director of Quality and Regulatory Affairs

13. ADDRESS *(Number, Street, State, and ZIP Code) (of person identified in Nos. 11 and 12)*
   ARK Diagnostics, Inc.
   1190 Bordeaux Drive
   Sunnyvale, CA 94089

14. TELEPHONE AND FAX NUMBERS *(Include Area Code)*
   (Tel.) 408-747-0708
   (Fax) 408-747-0783

15. DATE OF CERTIFICATION
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Analysis of Methotrexate and 7-Hydroxymethotrexate by High-Performance Liquid Chromatography and Preliminary Clinical Studies

Christine P. Collier, Stuart M. MacLeod, and Steven J. Soldin

Departments of Clinical Biochemistry and Pharmacology, University of Toronto, and Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada

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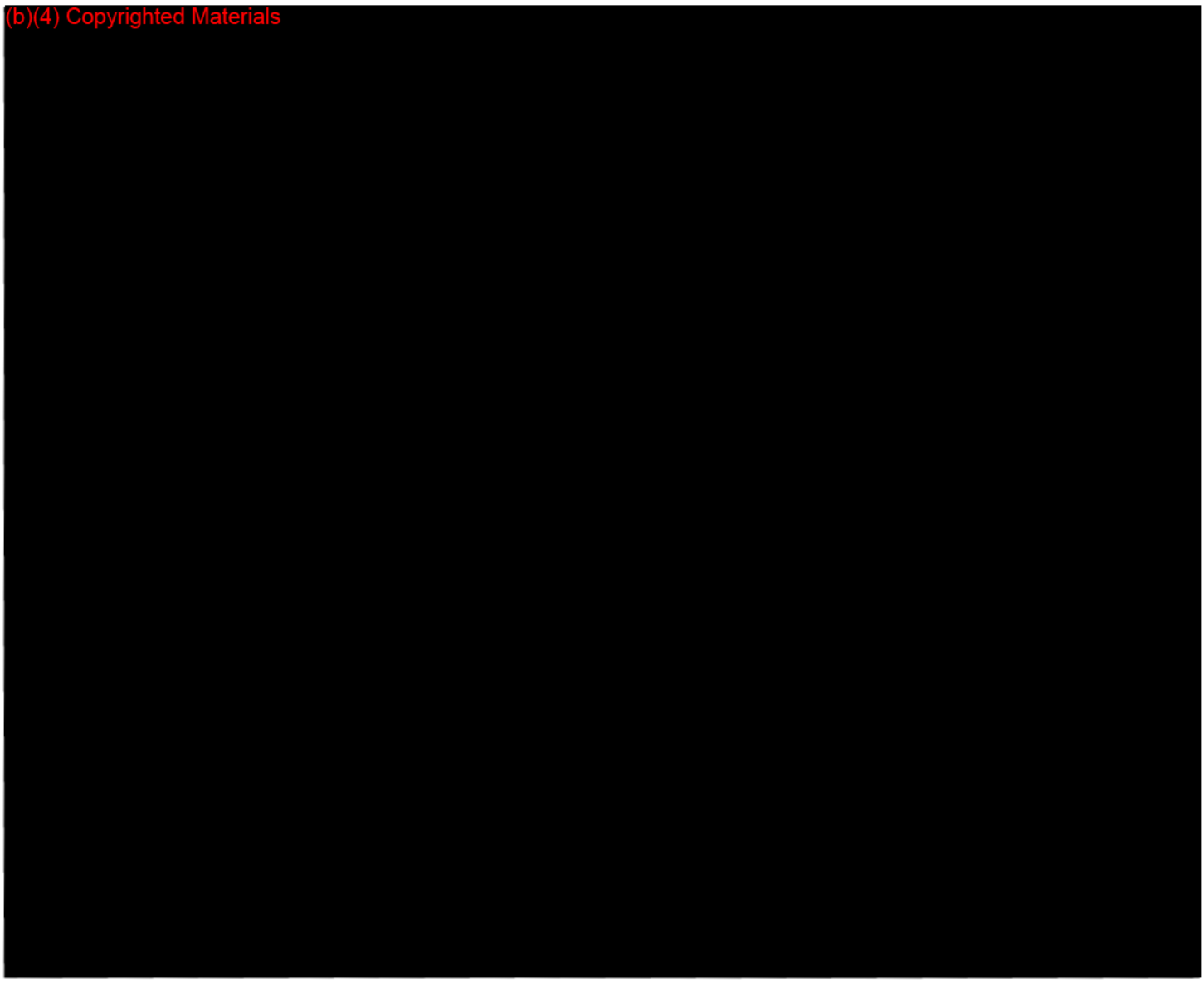

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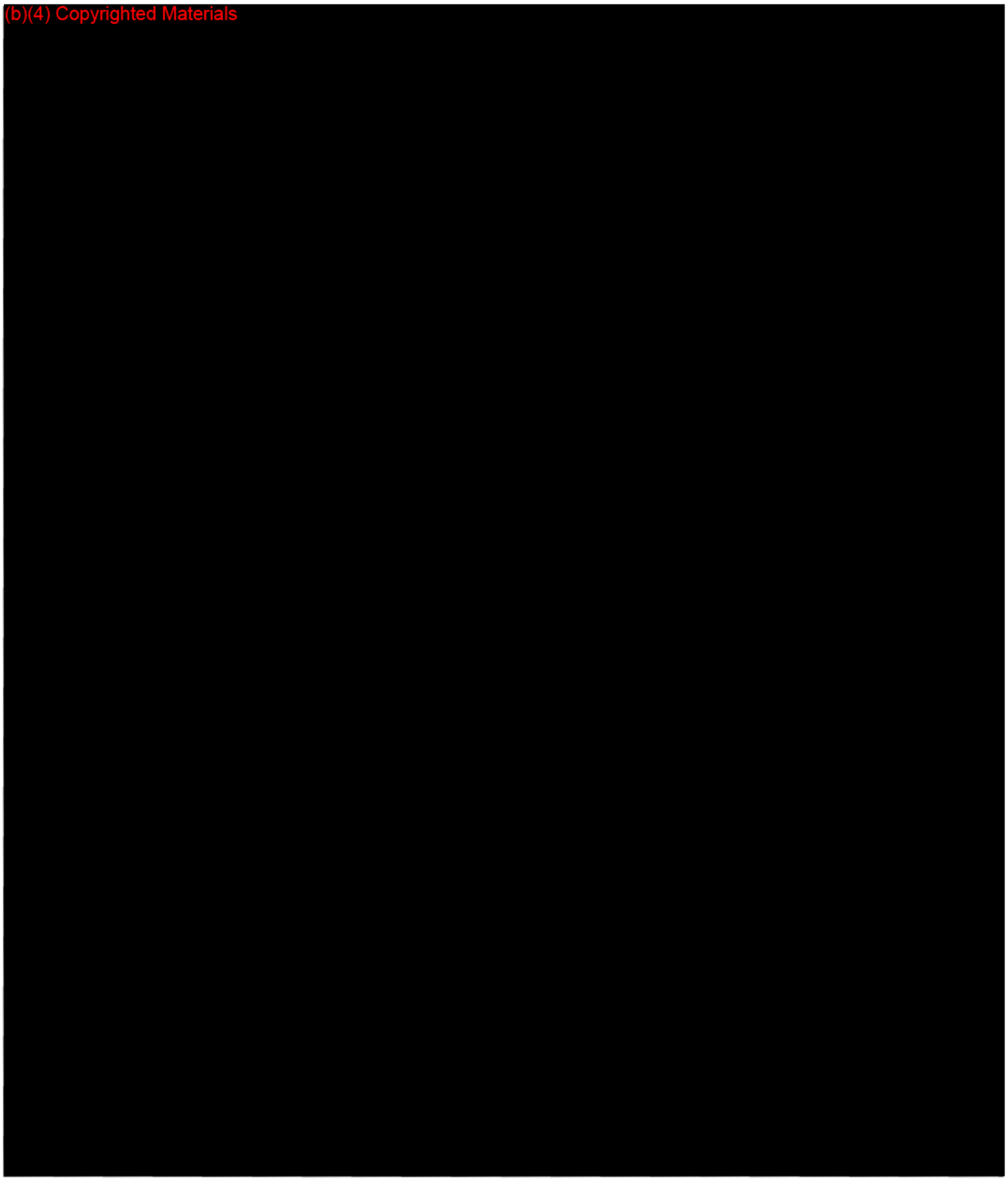

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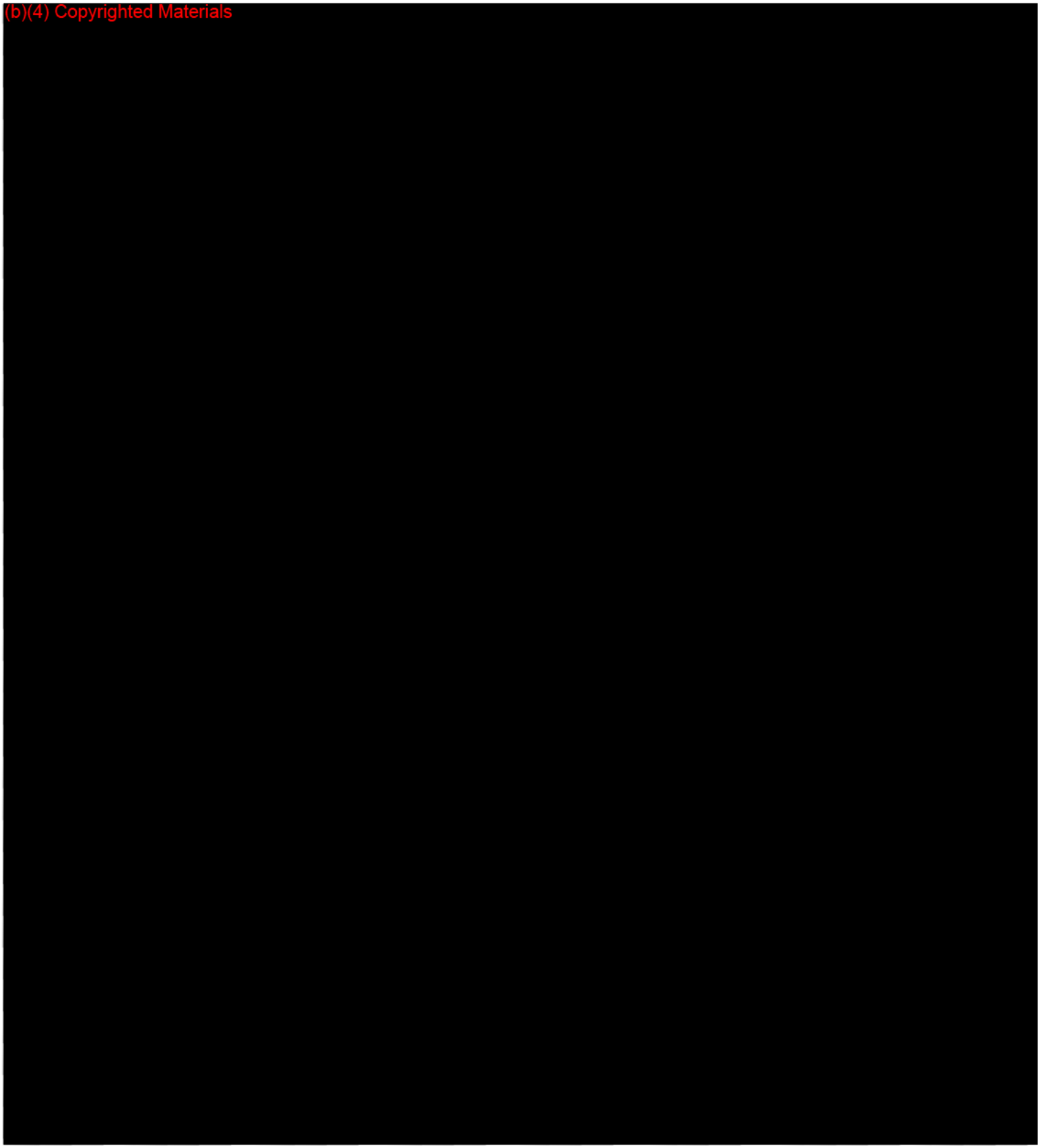

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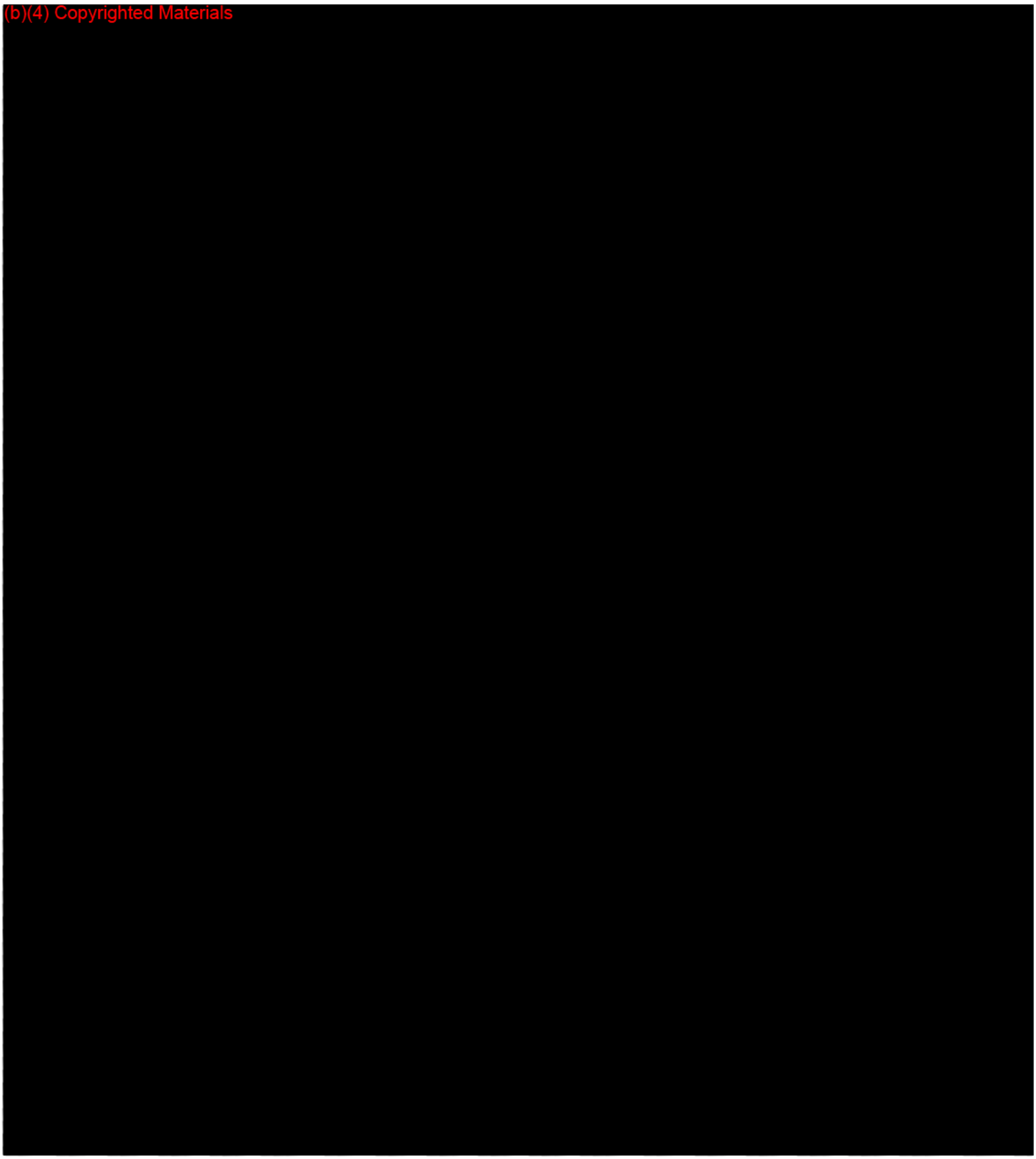

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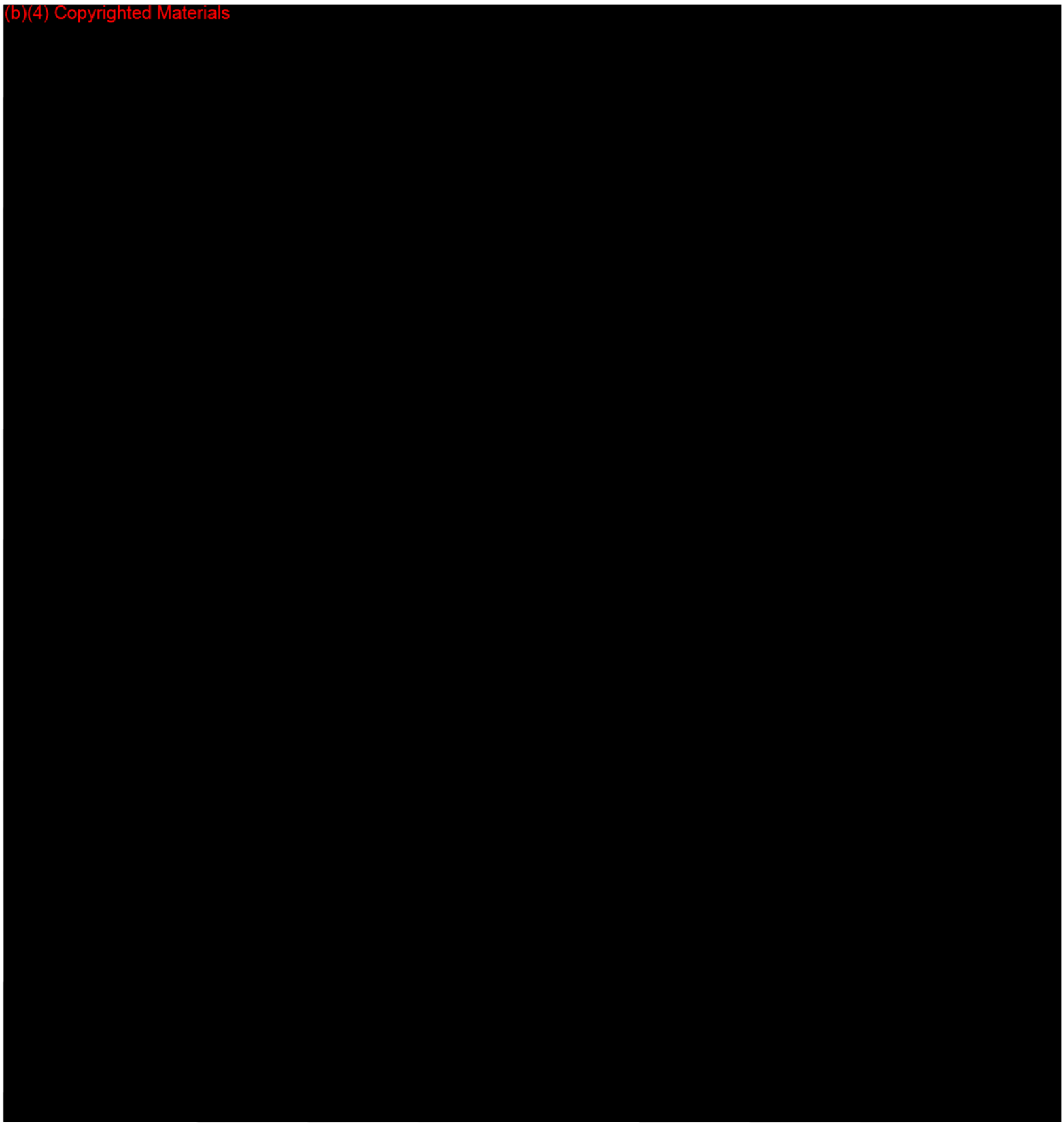

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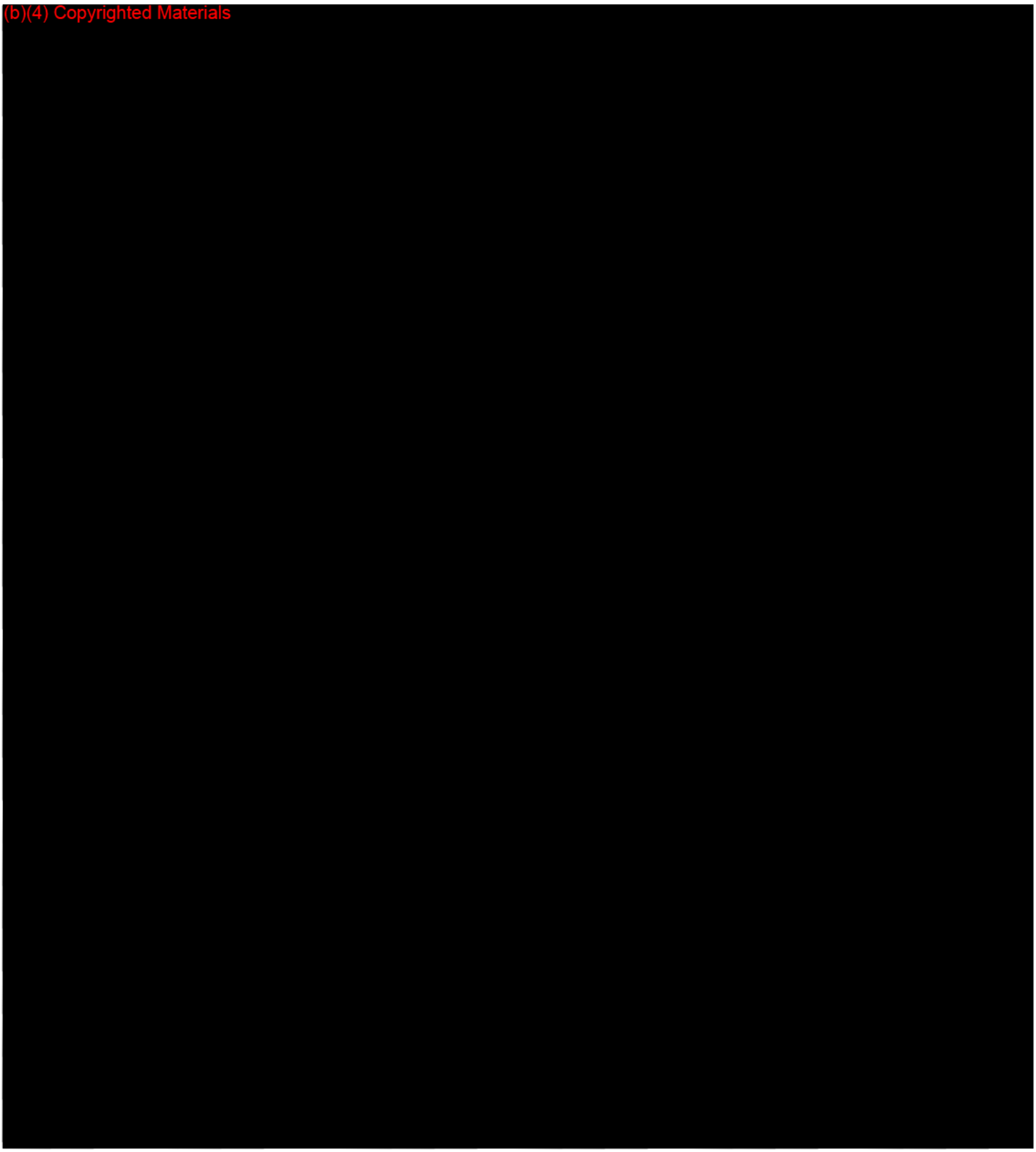

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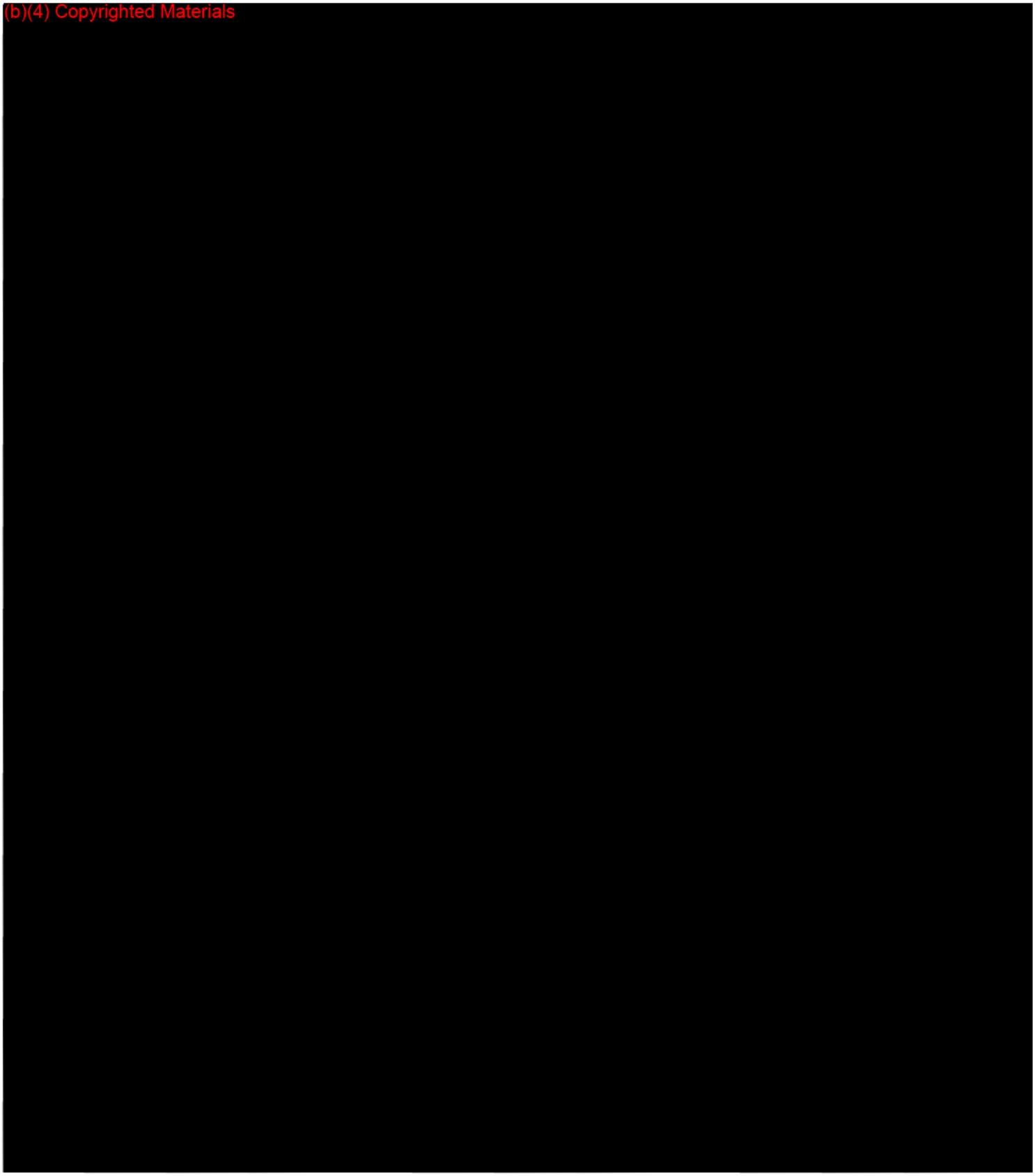

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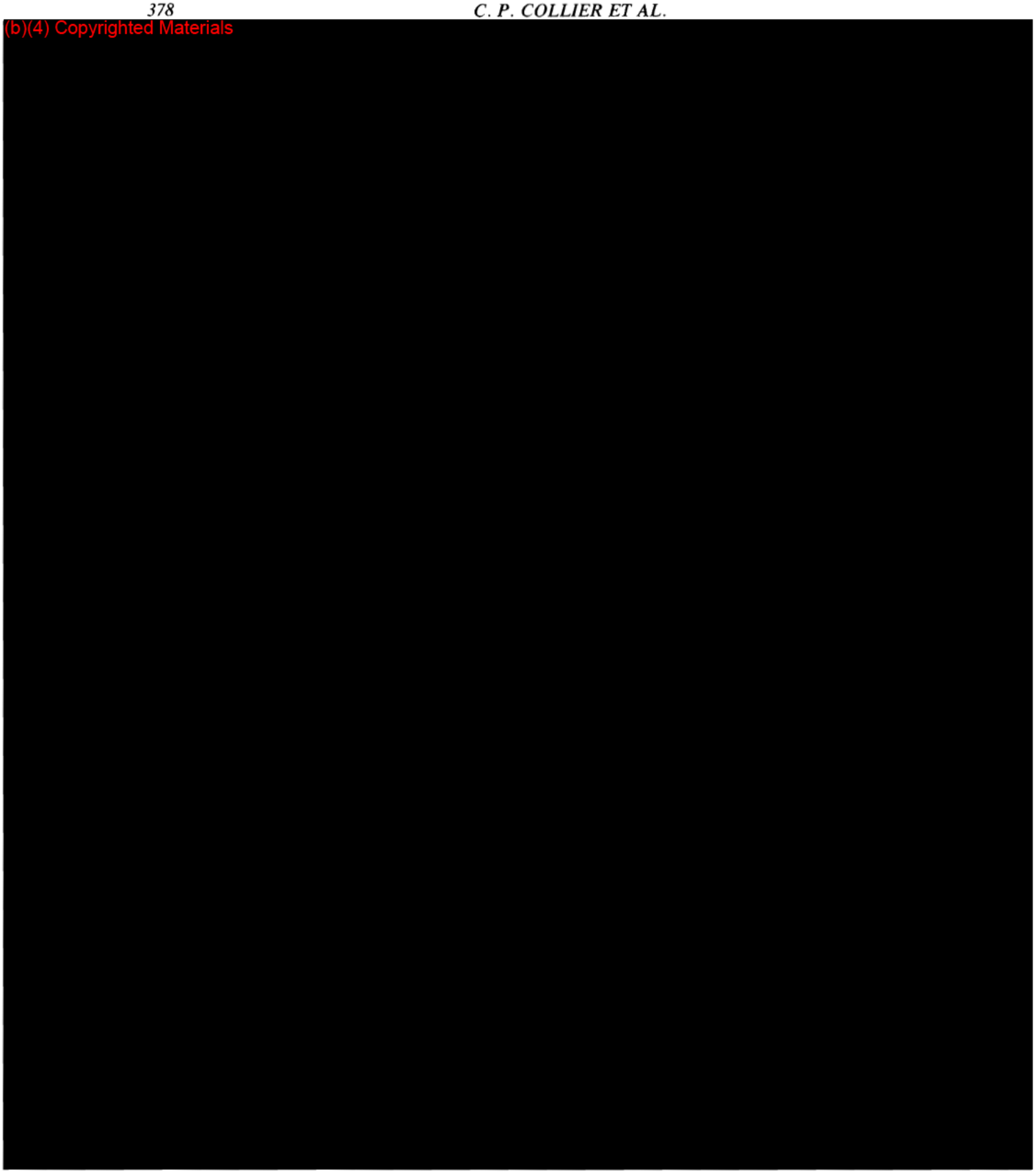

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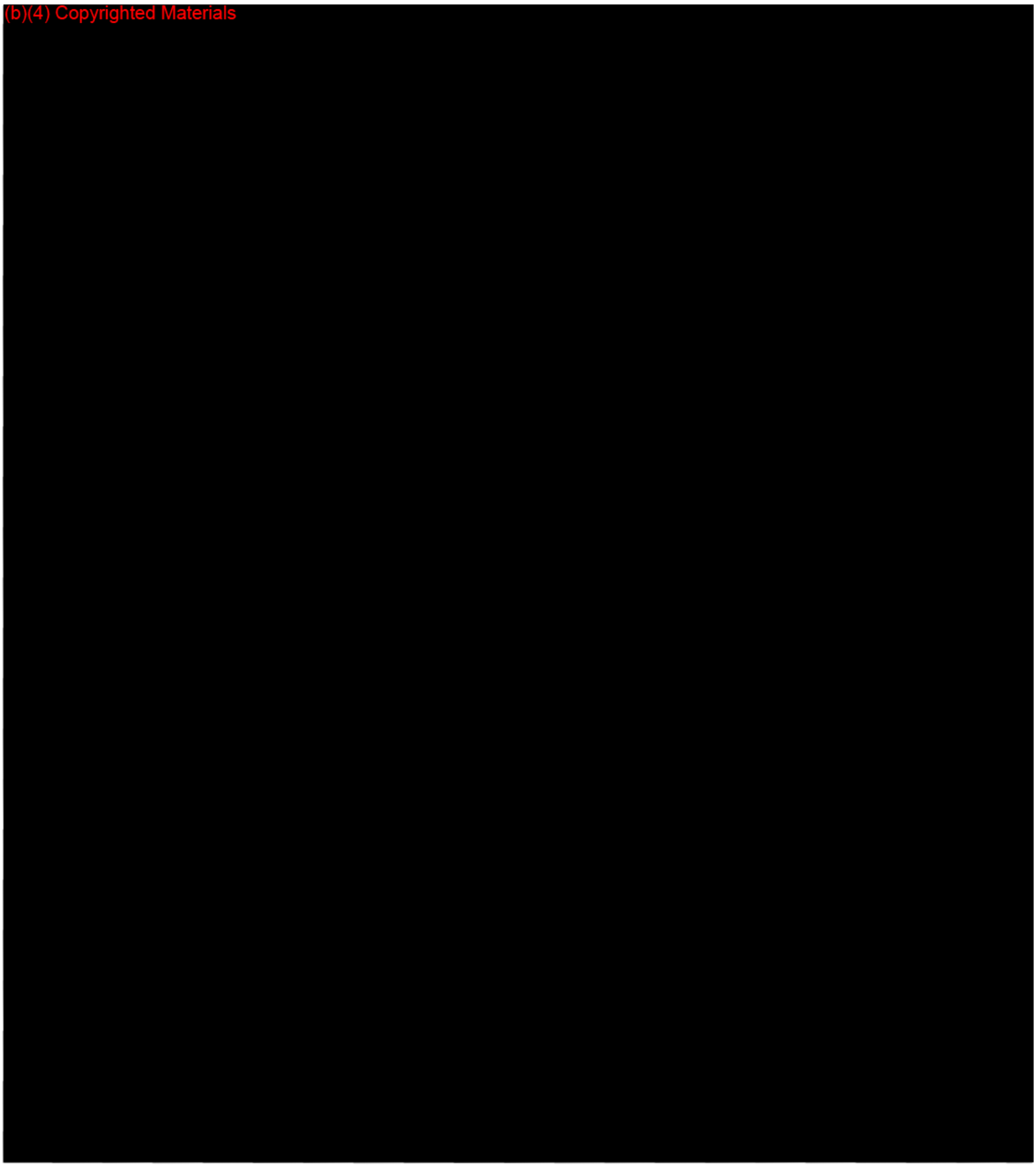

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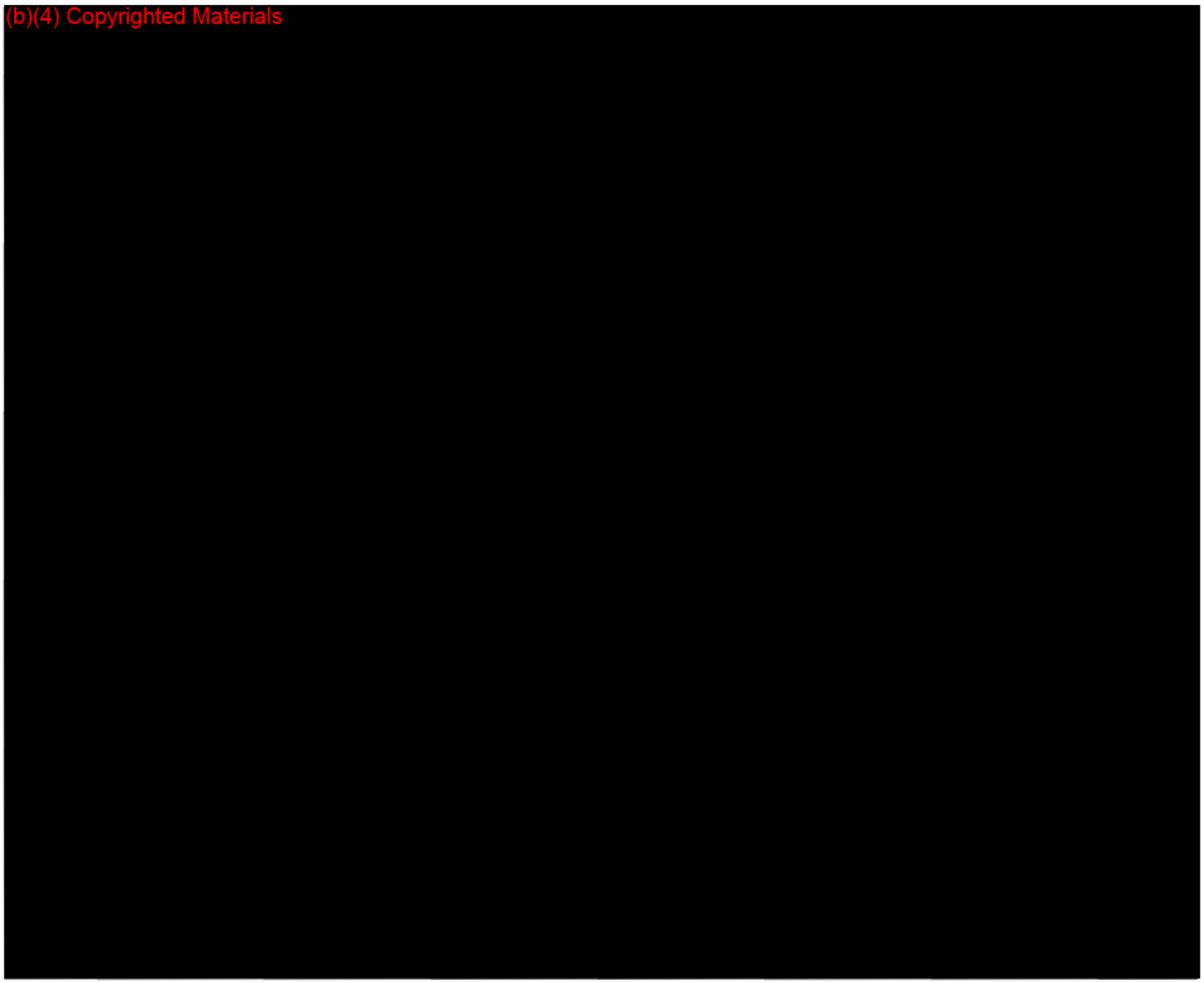

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Population Pharmacokinetics of High-Dose Methotrexate After Intravenous Administration in Pediatric Patients With Osteosarcoma

Helena Colom, PhD, Rosa Farré, PharmD, PhD,† Dolors Soy, PharmD, PhD,‡ Concepción Peraire, PhD,* Josep-Maria Cendros, PhD,* Nuria Pardo, MD,§ Montserrat Torrent, MD,§ Josep Domenech, PhD,* and Maria-Antonia Mangues, PharmD, PhD†*

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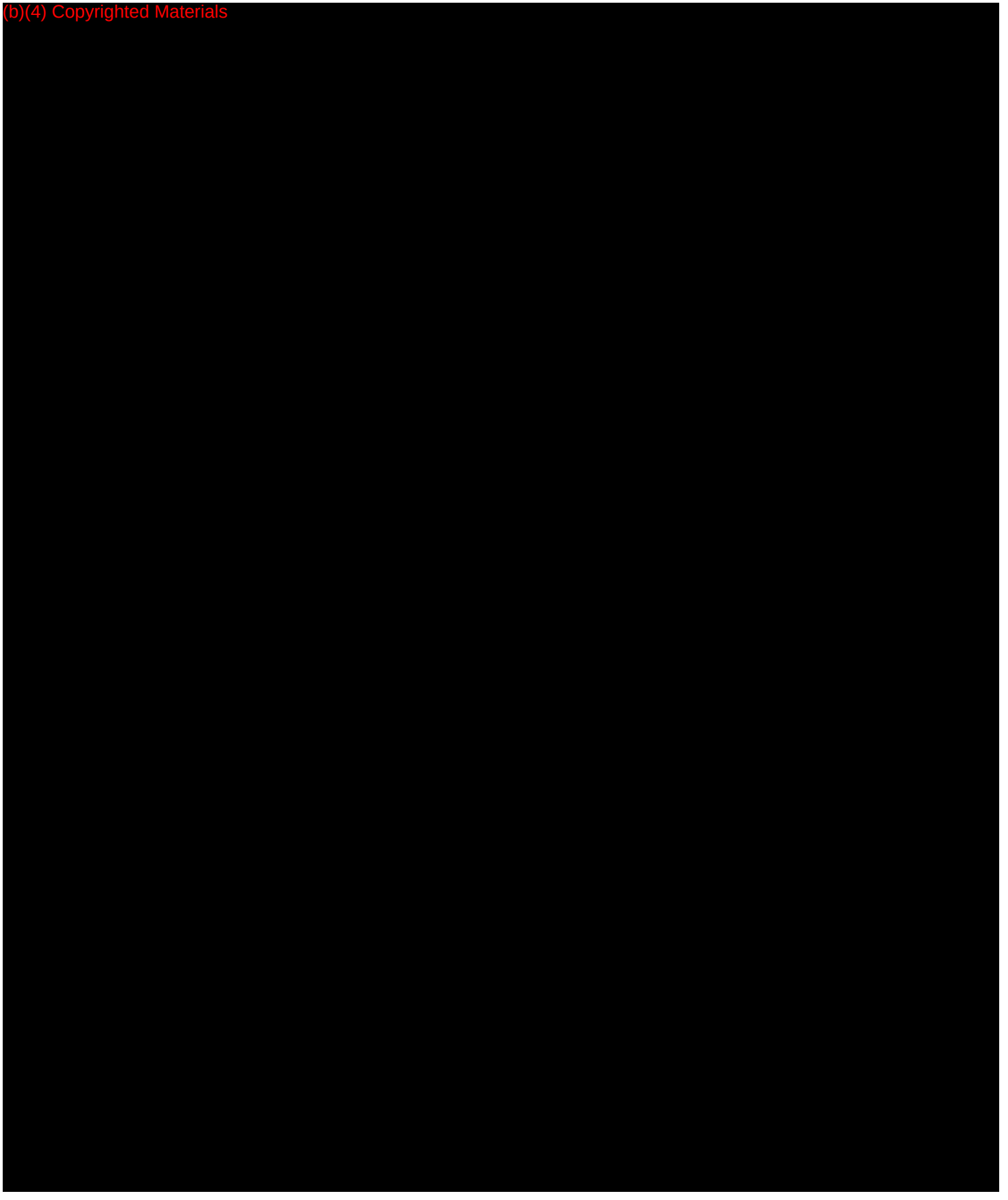

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Label Proof – Methotrexate Products

1. PURPOSE

This document comprises the label proofs for comparison of raw material stock labels for the Methotrexate assay products, reagents, calibrators, controls and dilution buffer. The label proofs are actual, approved pre-printed stock labels. It also comprises appended copies of the current approved version of package inserts to facilitate material inspection.

2. SCOPE

The process guideline applies to the inspection of raw material stock labels and package inserts for Methotrexate assay products at ARK Diagnostics, Inc (ARK).

3. REFERENCES

0740-0026-01 – Purchase Requisition for Methotrexate Package Inserts
0743-0001-00 – Receiving and Inspection
0743-0001-01 – Receiving Purchase Order - Inventoried Item
0900-0003-38 – Product Label Printing In-House
0900-0004-23 – Specification, ARK Methotrexate Package Inserts
0900-0004-24 – Label Proofs – Methotrexate Assay

4. RESPONSIBILITIES

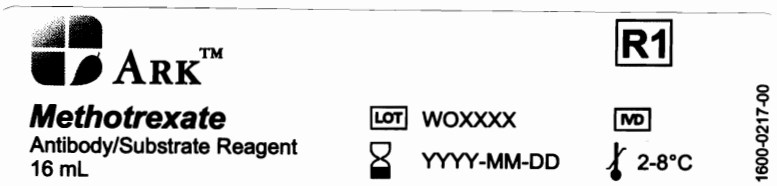
OPERATIONS and QA are responsible for maintaining this guideline.

5. PROCEDURE

5.1. Container Labels for Methotrexate assay products are produced according to Doc. No. 0900-0003-38 – Product Label Printing In-House. Compare the label for its respective product.

5.2. Consult this label proof for each label part number. Verify the lot number and expiration date of produced labels according to the Production Order and printing procedure. This label proof is comprised of labels with generic lot number **WOXXXX** and expiration date **YYYY/MM/DD**.

5.3. Bottle Labels for Methotrexate Reagents



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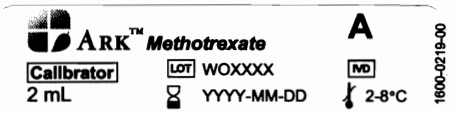
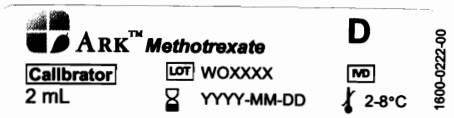
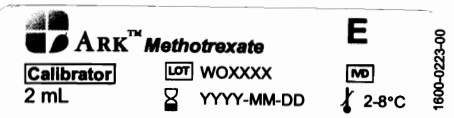
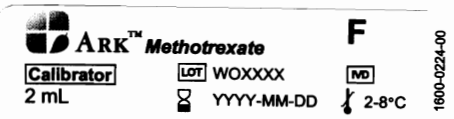
Label Proof – Methotrexate Products

5.4. Bottle Labels for Methotrexate Calibrators

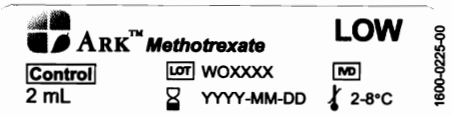


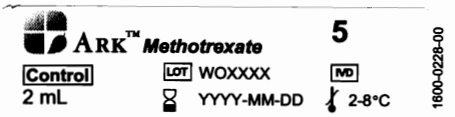


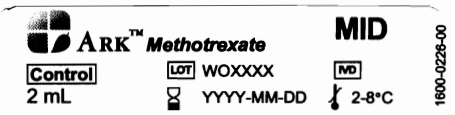


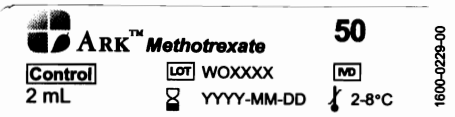


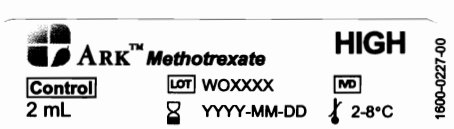
5.5. Bottle Labels for Methotrexate Controls











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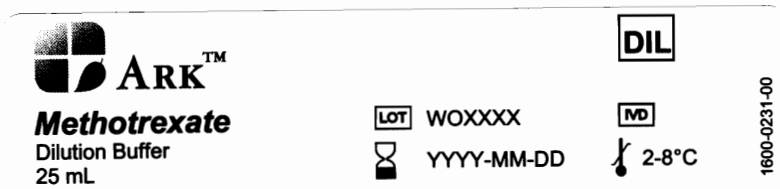
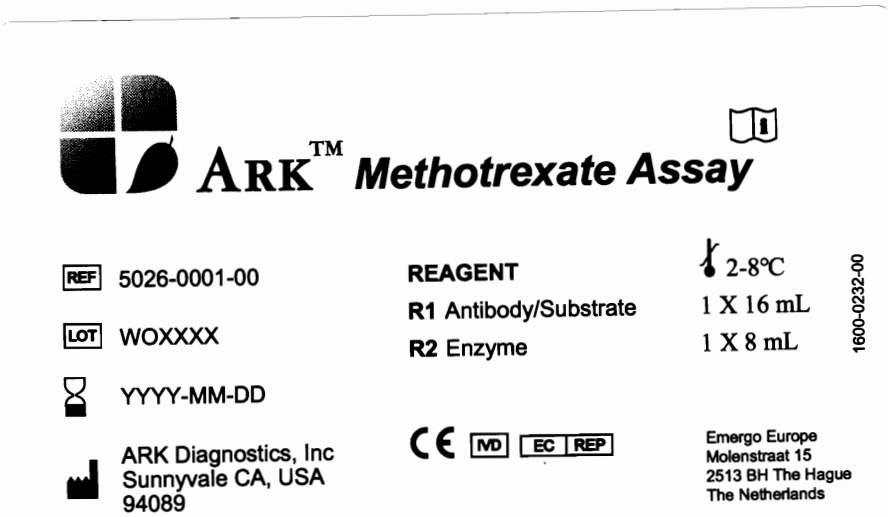
Label Proof – Methotrexate Products

5.6. Bottle Label for Methotrexate Dilution Buffer



5.7. Kit Label for Methotrexate Reagents



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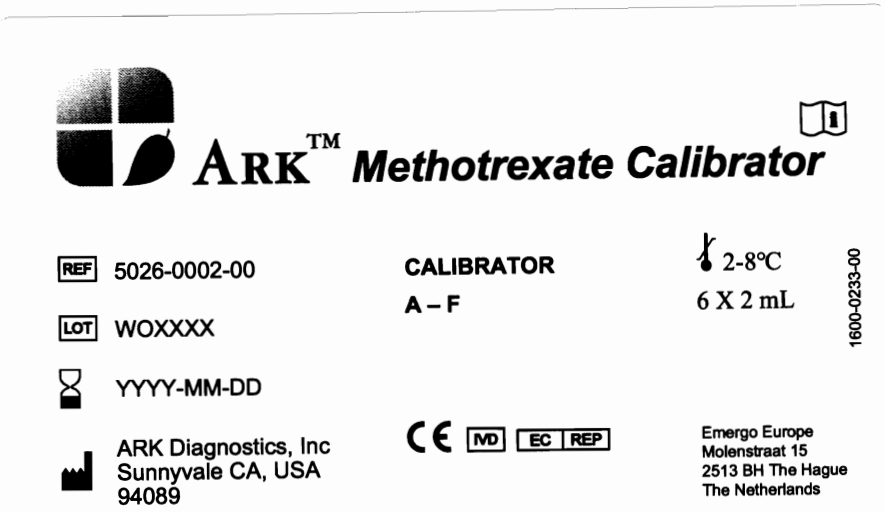
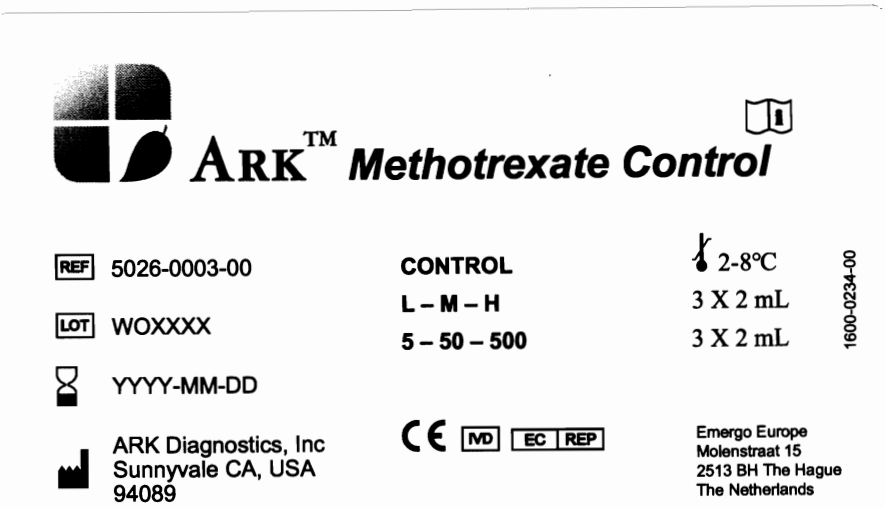
Label Proof – Methotrexate Products

5.8. Kit Label for Methotrexate Calibrators



5.9. Kit Label for Methotrexate Controls [complete set]



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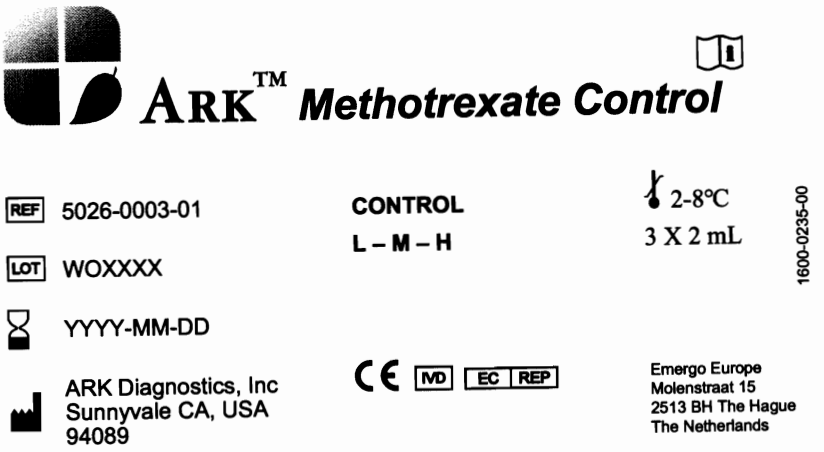
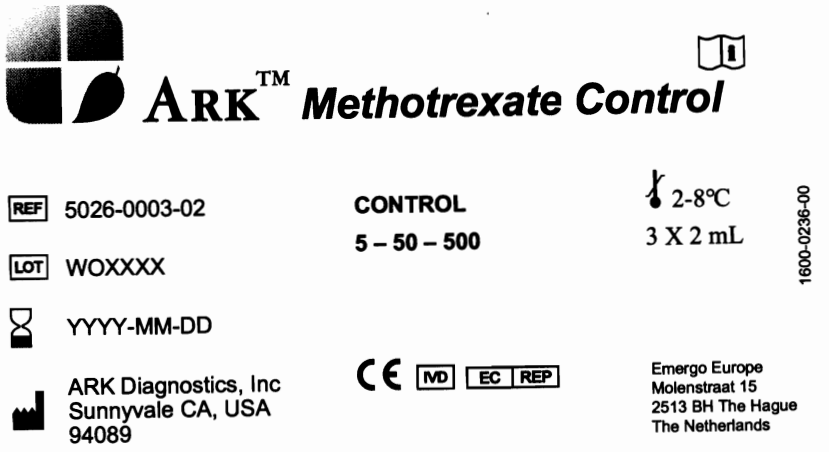
5.10. Kit Label for Methotrexate Controls [Calibration Range set]



5.11. Kit Label for Methotrexate Controls [High Range set]



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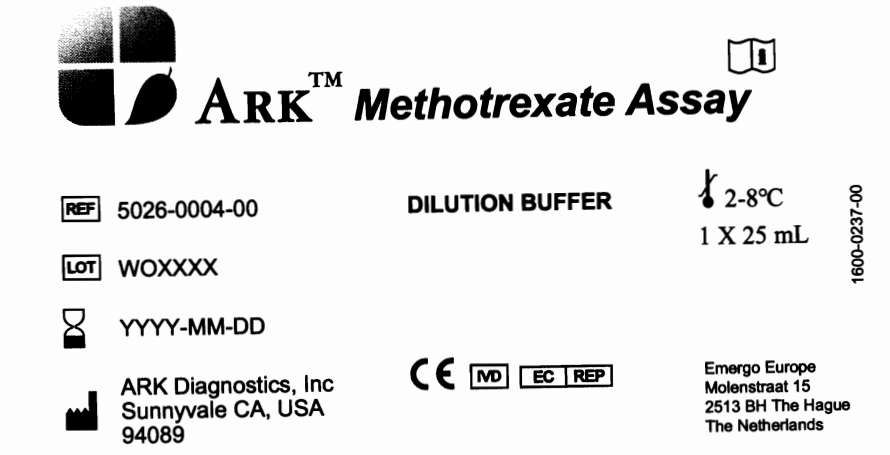
Label Proof – Methotrexate Products

5.12. Kit Label for Methotrexate Dilution Buffer



5.13. Appended copies of package inserts:

- 1600-0213-00 – June 2011, Rev01, Methotrexate Assay
- 1600-0214-00 – June 2011, Rev01, Methotrexate Calibrator
- 1600-0215-00 – June 2011, Rev01, Methotrexate Control
- 1600-0216-00 – June 2011, Rev01, Methotrexate Dilution Buffer

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Evaluating Performance of a Decision Support System to Improve Methotrexate Pharmacotherapy in Children and Young Adults With Cancer

Erin Dombrowsky, MSE, Bhuvana Jayaraman, MPH, Mahesh Narayan, MB, MSE, and Jeffrey S. Barrett, PhD, FCP

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J Cancer Res Clin Oncol (1985) 109:86–88
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Cancer Research Clinical Oncology
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Short Communication

7-Hydroxy-Methotrexate and Clinical Toxicity Following High-Dose Methotrexate Therapy

R. Erttmann, S. Bielack, and G. Landbeck

Department of Hematology and Oncology Children's Hospital, University of Hamburg, Martinistr. 52, D-2000 Hamburg 20, Federal Republic of Germany

Summary. 7-Hydroxy-MTX production after consecutive high-dose MTX therapy (12 g/m^2) was measured in 7 patients with osteosarcoma by HPLC.

7-Hydroxy-MTX serum values in the last cycle were found to be significantly lower compared with the first high-dose MTX treatment of the adjuvant chemotherapy protocol (COSS 80).

Moreover, in another patient highly reduced 7-hydroxy-MTX production was correlated with severe clinical toxicity.

As 7-hydroxy-MTX is a 200 fold less potent dihydrofolic acid reductase inhibitor compared with MTX decreased production of the metabolite may lead to enhanced clinical toxicity which may not be predictable monitoring MTX serum levels alone.

Key words: High-dose MTX – MTX metabolism – 7-Hydroxy-MTX

Patients undergoing high-dose methotrexate (MTX) therapy (> 30 mg/kg) excrete significant amounts of the metabolite 7-hydroxy-methotrexate (7-OH-MTX) [1]. As the aqueous solubility of this metabolite is three- to fivefold less than that of MTX, 7-OH-MTX may contribute to the renal toxicity of the high-dose MTX regimen. Therefore, forced alkaline, diuresis is mandatory after high-dose MTX infusion. On the other hand, 7-OH-MTX is 200-fold less potent as an inhibitor of dihydrofolic acid reductase [2]. Moreover, 7-OH-MTX inhibits the transport of MTX into tumor cells in vitro as shown by Lankelma et al. [3]. 7-OH-MTX is excreted in amounts of up to 33% in terms of given MTX after high-dose therapy [1, 4, 5]. The 7-OH-MTX plasma levels exceed those of MTX by up to 100-fold 12–48 h after MTX administration [1, 4, 5]. In conclusion, generation of 7-OH-MTX after high-dose MTX therapy may contribute to the rescue of tissue from MTX intoxication.

We measured 7-OH-MTX and MTX concentration in plasma after administering a high dose of MTX (12 g/m^2), followed by "leukovorin rescue" and forced alkaline diuresis, in seven patients with osteosarcoma by reversed phase high-performance liquid chromatography (HPLC) [6]. Fourteen drug cycles of high-dose MTX therapy were part of our adjuvant chemotherapy protocol (COSS-80) [7].

As shown in Fig. 1, there is a marked difference in the molar quotient of 7-OH-MTX to MTX between the first and last MTX drug cycle. This was not due to delayed MTX excretion. Therefore, it may be con-

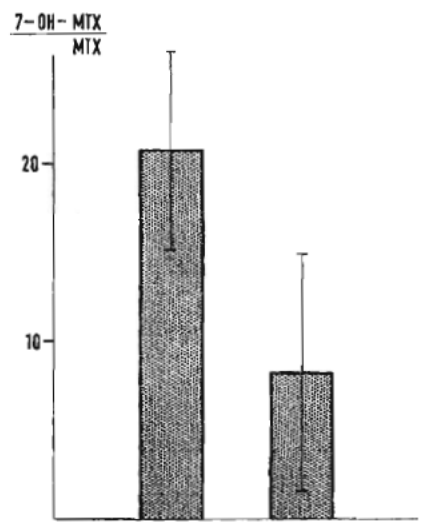

Fig. 1. Comparison of 7-OH-MTX to MTX ratio between the first and last high-dose MTX cycle of the COSS-80 chemotherapy protocol. The values are presented as mean ± SD of 7-OH-MTX to MTX molar ratio obtained 12 h after MTX infusion. Seven patients. MTX dosage 12 g/m^2 body surface. The difference was significant according to the Wilcoxon test ($\alpha = 0.01$). The absolute 7-OH-MTX and MTX values of each patient are given in Table I

Offprint requests to: R. Erttmann (address see above)

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Table 1. 7-OH-MTX and MTX levels of 7 patients undergoing high-dose MTX therapy

	Patient no.						
	1	2	3	4	5	6	7
First drug cycle							
7-OH-MTX	820[a]	877	1,013	658	1,408	1,898	268
MTX	85.7	37.1	41.1	22.6	18.2	81.1	14.5
Last drug cycle							
No. of cycle	10	13	12	14	10	14	10
7-OH-MTX	414	376	191	359	1,132	295	507
MTX	62.6	40.2	42.2	62.2	19,8	94.5	20.8

[a] Values are given as micromoles per liter

cluded that cumulative hepatic toxicity of chemotherapy may reduce MTX metabolism. This may lead to diminished "endogenous rescue" by 7-OH-MTX. The absolute values of 7-OH-MTX and MTX in the first and last drug cycle are given in Table 1. Our preliminary results could explain the observation that patients receiving more than ten infusions of high-dose MTX experienced symptoms of toxicity significantly more frequently than those receiving fewer infusions [8].

This assumption is confirmed by another observation in a patient with osteosarcoma who showed severe stomatitis, bone marrow aplasia, and hepatoxicity following high-dose MTX therapy beginning after the ninth drug cycle. As shown in Fig. 2, there is a close relation between observed toxicity and decreasing production of 7-OH-MTX, indicated by the molar quotient of 7-OH-MTX to MTX plasma level measured 12 h after MTX infusion. Beginning with the ninth MTX cycle, the 7-OH-MTX to MTX quotient dropped to values of about 1, which was followed by clinical toxicity. This was not due to delayed MTX excretion. As MTX values were $< 10^{-7}$ M 48 h after MTX infusion (with the exception of cycle 12), "leukovorin rescue" was not enhanced or prolonged. The absolute values of 7-OH-MTX and MTX 12 h after MTX infusion are given in Table 2.

In conclusion, the observations presented here raise the question of whether MTX metabolism leading to 7-OH-MTX production contributes to "endogenous" rescue from MTX toxicity. The monitoring of MTX in patients plasma for the early detection of those with a high risk of toxicity (who will require increased dose and prolonged course of leukovorin) is mandatory.

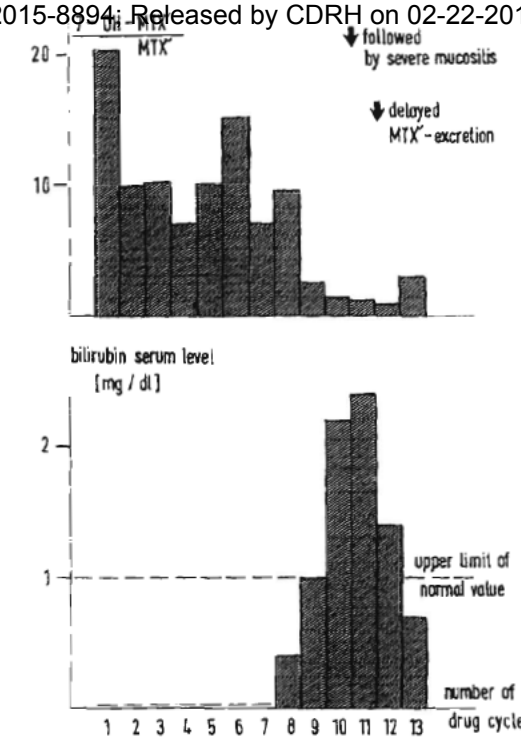

Fig. 2. 7-OH-MTX to MTX ratio in consecutive high-dose MTX drug cycles of a patient with osteosarcoma undergoing the COSS-80 chemotherapy protocol. Beginning with the ninth drug cycle, 7-OH-MTX production falls, which correlates with a rising serum bilirubin level, severe mucositis, and bone marrow aplasia. The absolute 7-OH-MTX and MTX levels are given in Table 2

As the results presented show, decreased levels of 7-OH-MTX may be another parameter indicating an enhanced risk for MTX toxicity not detectable by measuring MTX plasma levels alone.

Further studies on this question are required. In reporting our preliminary results, we hope to stimulate others treating patients with high-dose MTX therapy to look for the clinical role of MTX metabolism.

Table 2. 7-OH-MTX and MTX levels in consecutive MTX drug cycles of a patient with osteosarcoma

	No. of drug cycle												
	1	2	3	4	5	6	7	8	9	10	11	12	13
7-OH-MTX	1,898[a]	701	412	318	327	333	384	325	168	70.7	84.7	257	295
MTX	82.6	40.2	42.2	62.2	19,8	94.5	20.8	85.7	37.1	41.1	22.6	18.2	94.5

[a] Values are given as micromoles per liter. Further details on the clinical course of this patient are given in Fig. 2

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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Received July 12, 1984/Accepted September 28, 1984

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

High-Dose Leucovorin as Sole Therapy for Methotrexate Toxicity

By Carlos D. Flombaum and Paul A. Meyers

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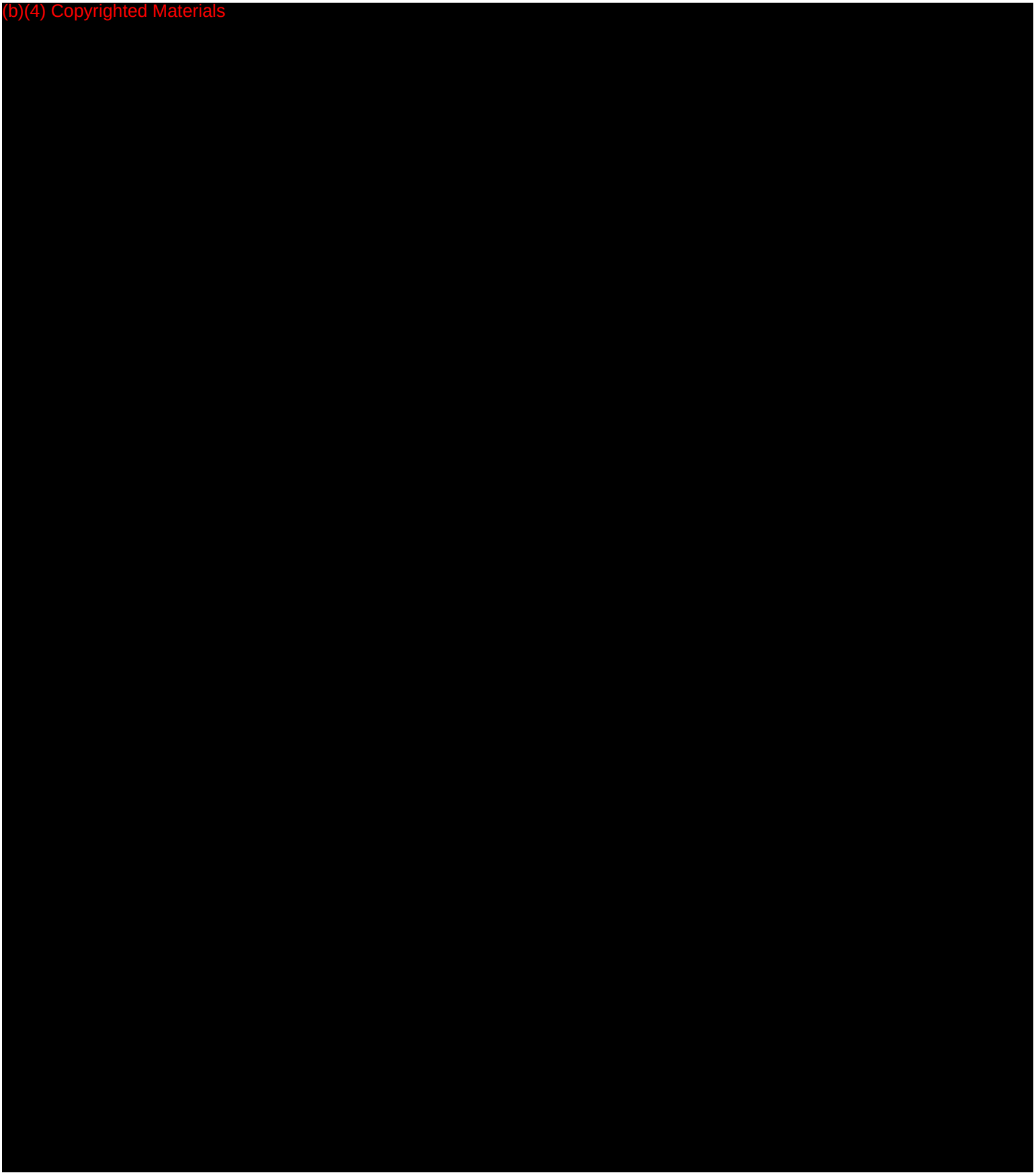

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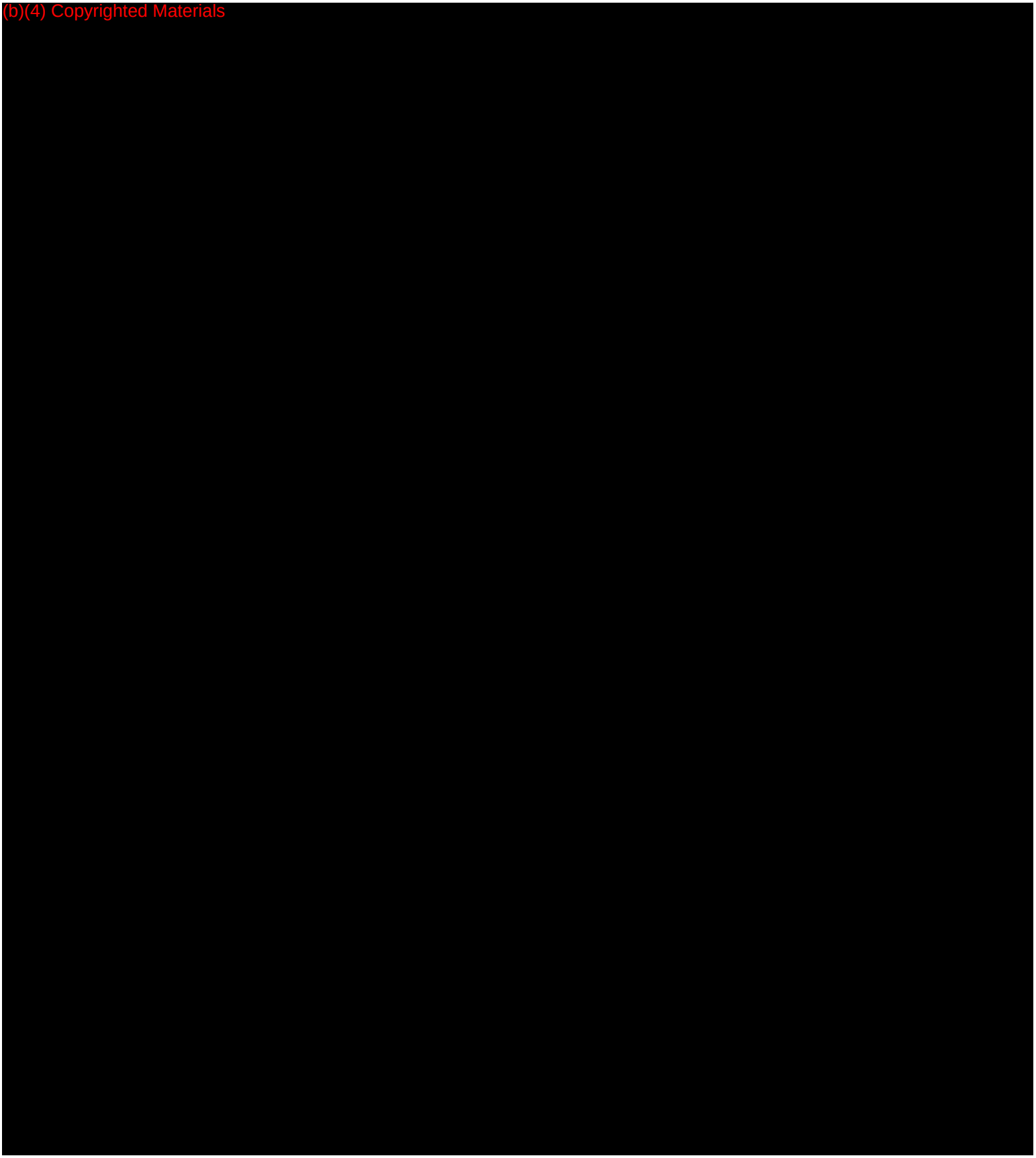

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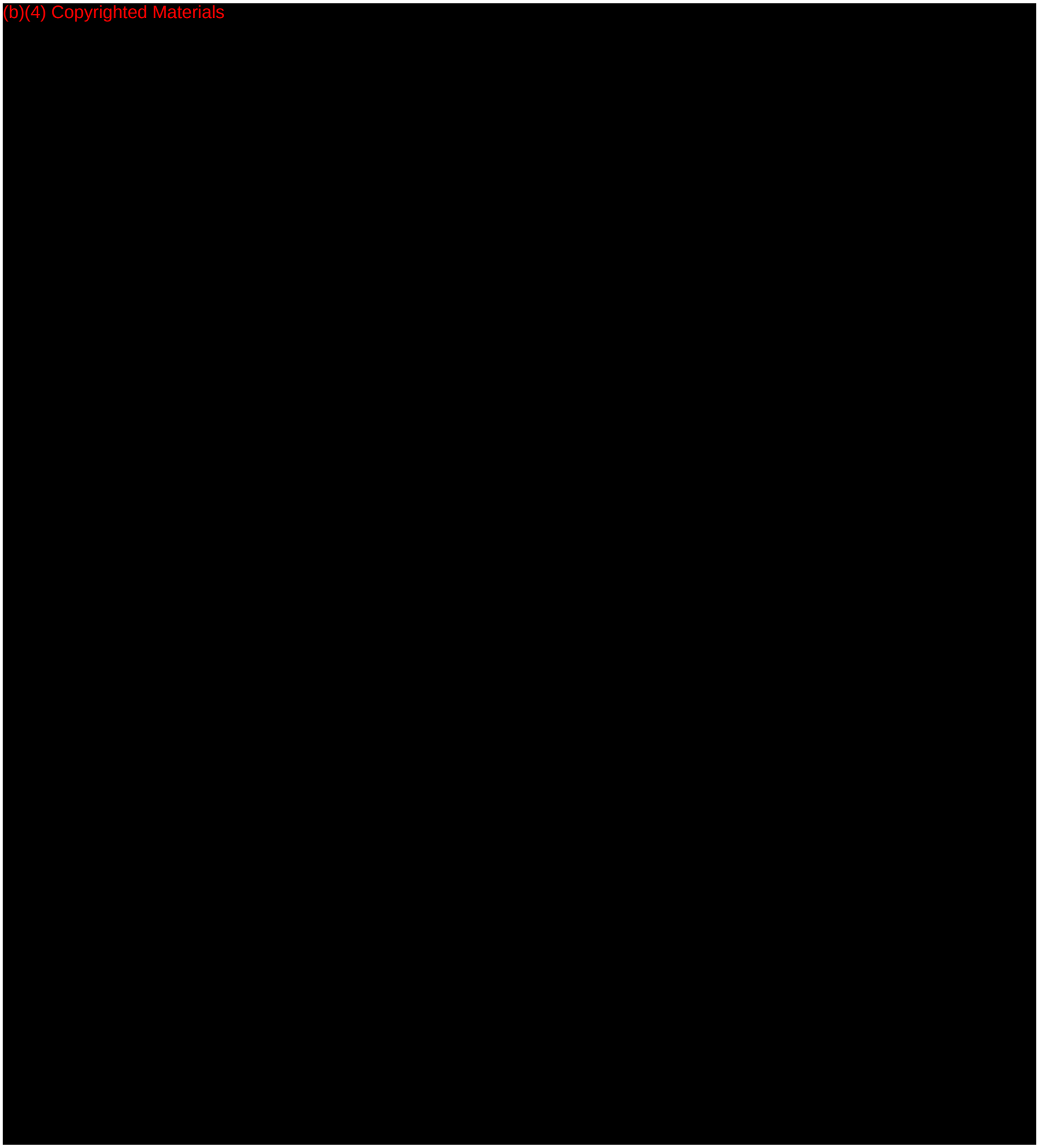

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British Journal of Haematology, 1990, **76**, 359–364

'MACHO' chemotherapy for stage IV B cell lymphoma and B cell acute lymphoblastic leukaemia of childhood

I. M. HANN,[1] O. B. EDEN,[2] J. BARNES,[3] C. R. PINKERTON[4] on behalf of the United Kingdom Children's Cancer Study Group (UKCCSG) [1]*Hospitals for Sick Children, Great Ormond Street, London WC1N 3JH,* [2]*Royal Hospital for Sick Children, 17 Millerfield Place, Edinburgh EH9 1LF,* [3]*UKCCSG Trials Office, Leicester LE1 7RH,* [4]*Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT*

Received 29 December 1989; accepted for publication 20 June 1990

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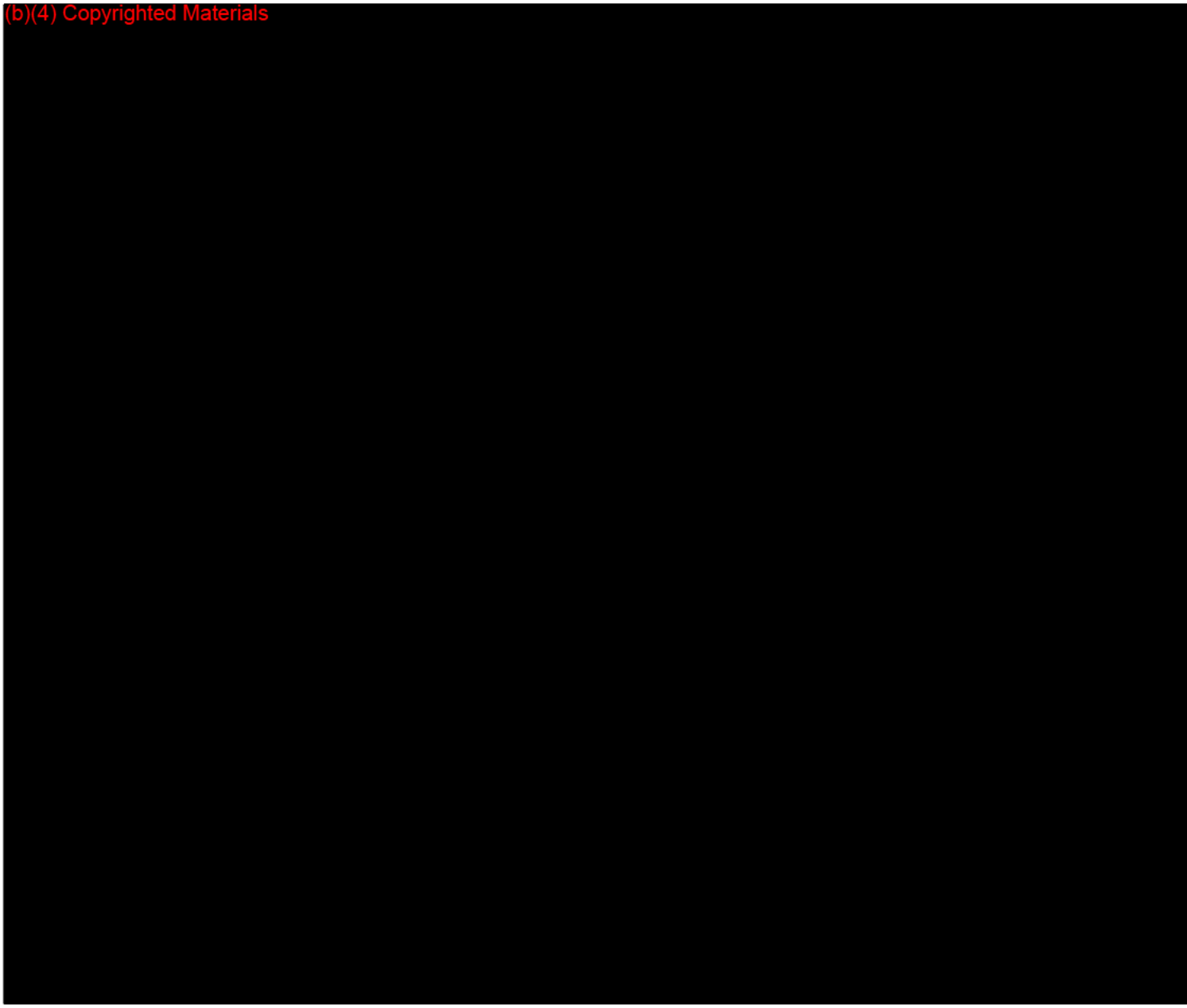

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Elsevier

Research Papers

Kinetics of degradation of methotrexate in aqueous solution

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(Received October 5th, 1982)
(Modified version received February 5th, 1983)
(Accepted February 11th, 1983)

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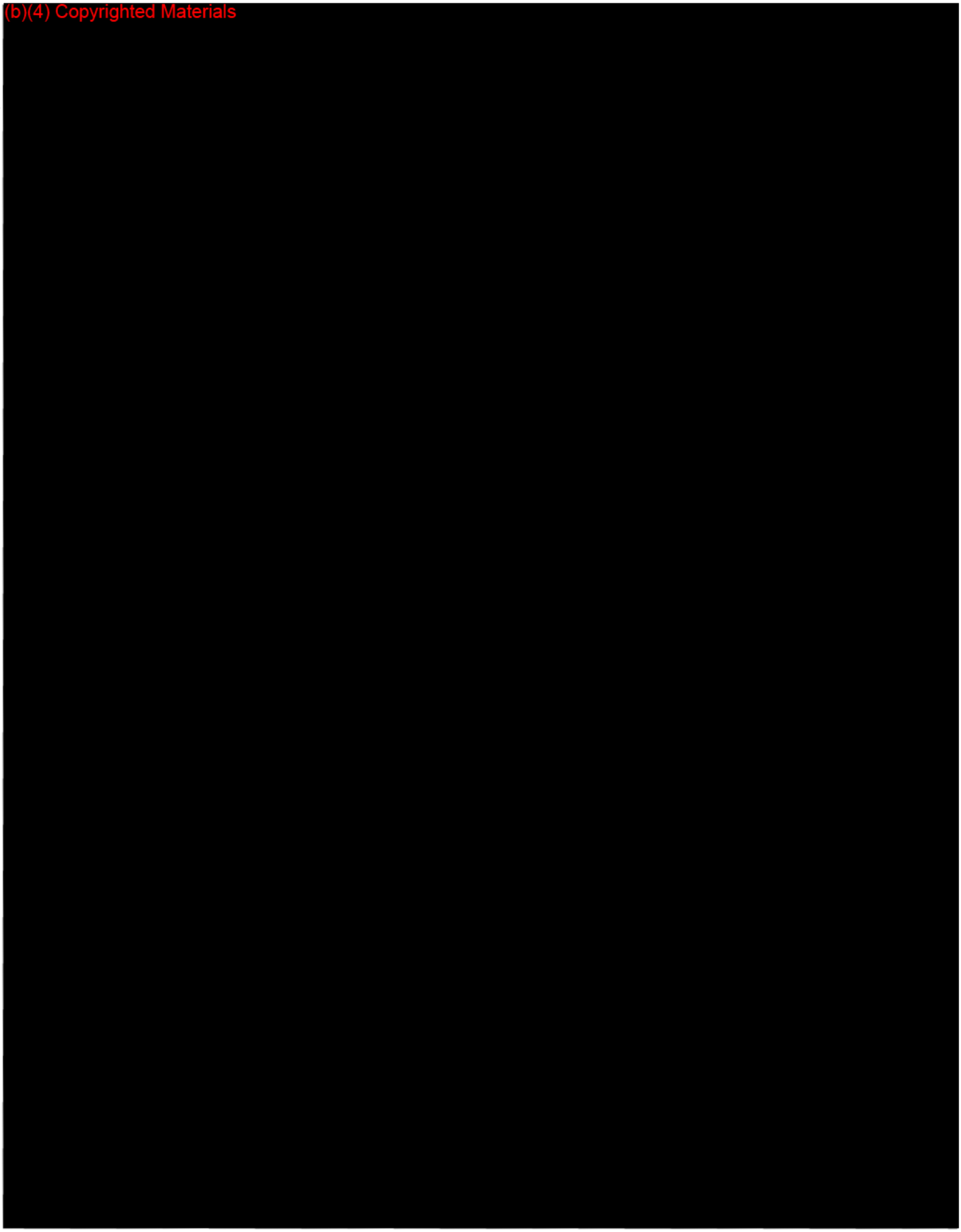

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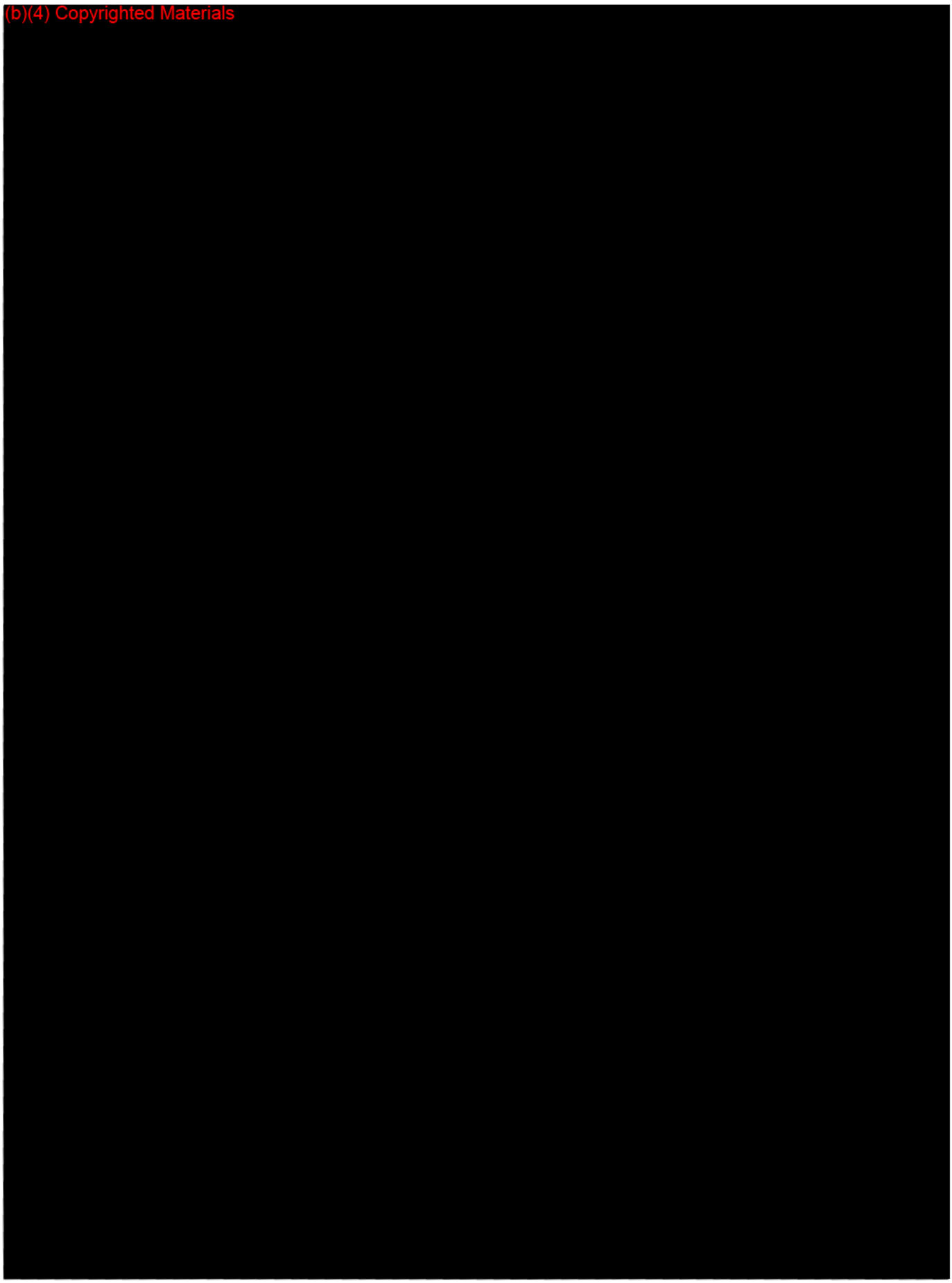

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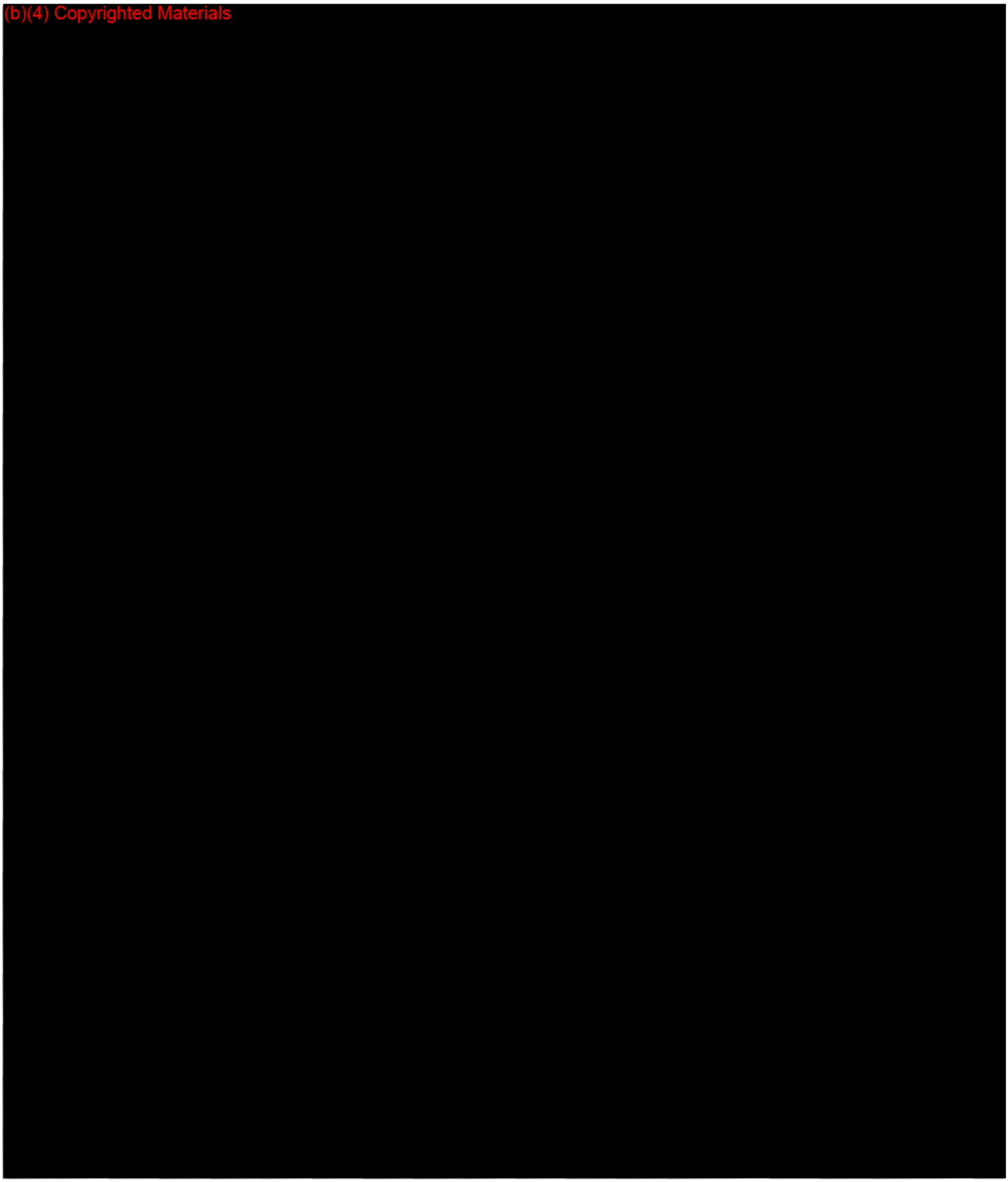

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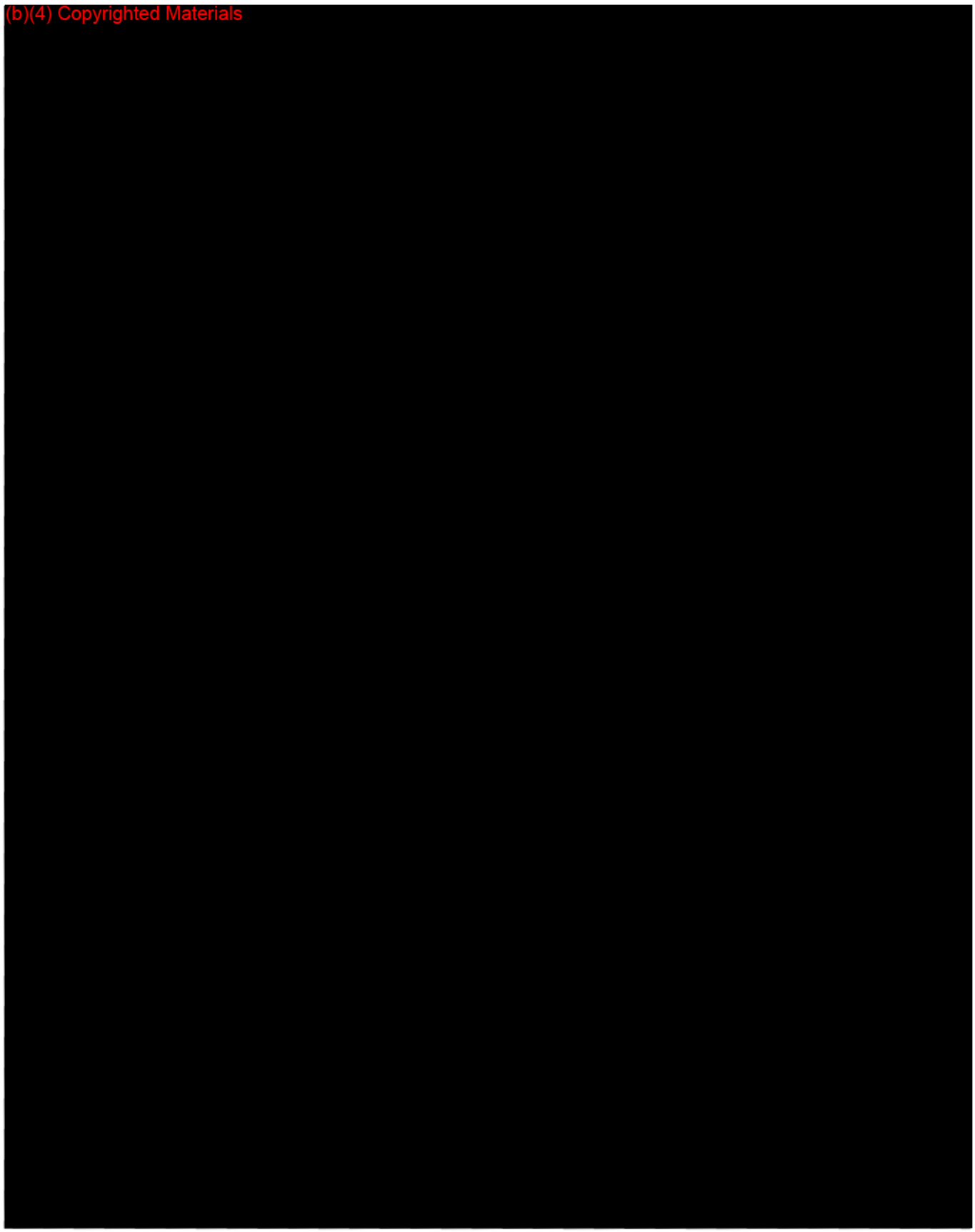

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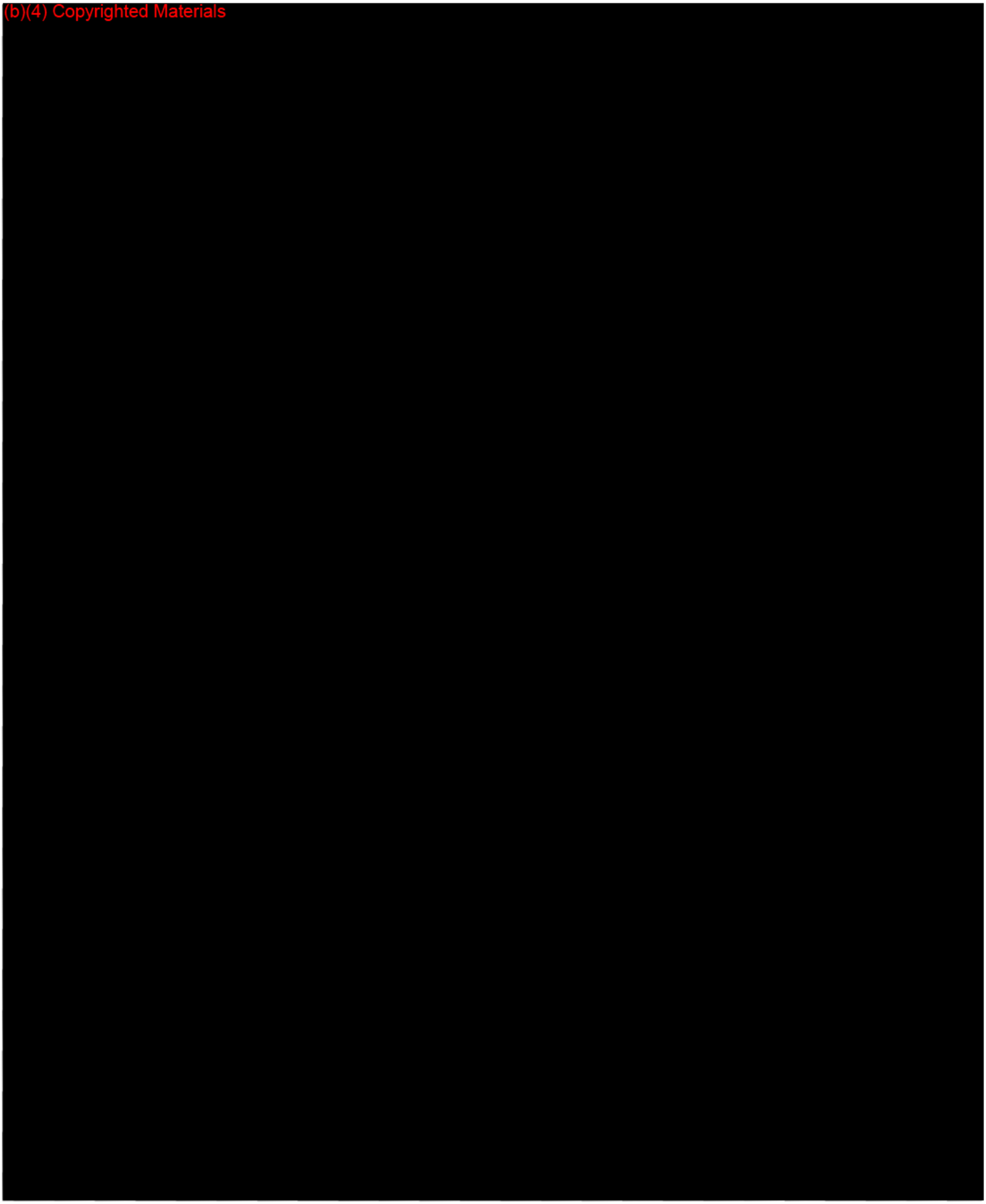

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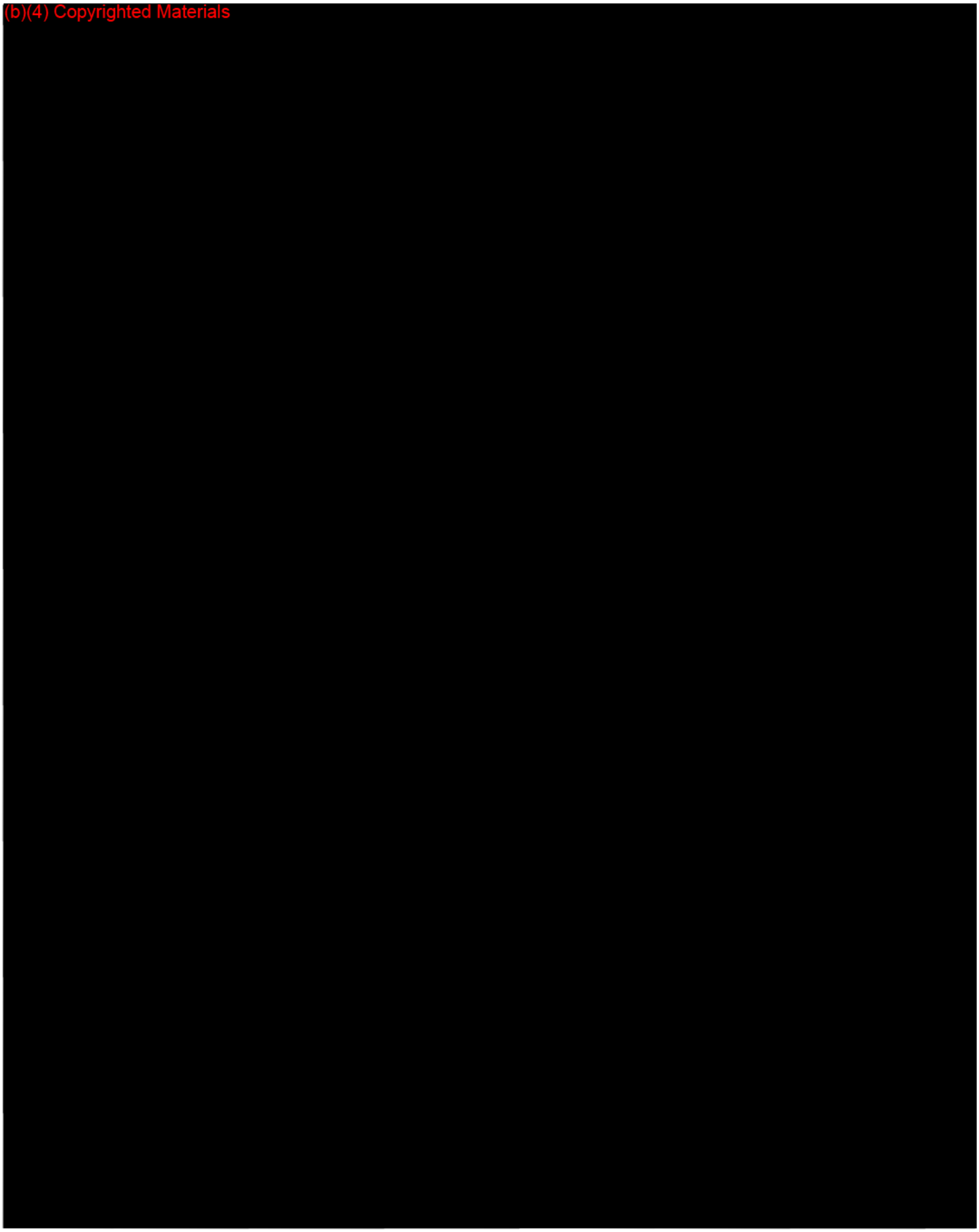

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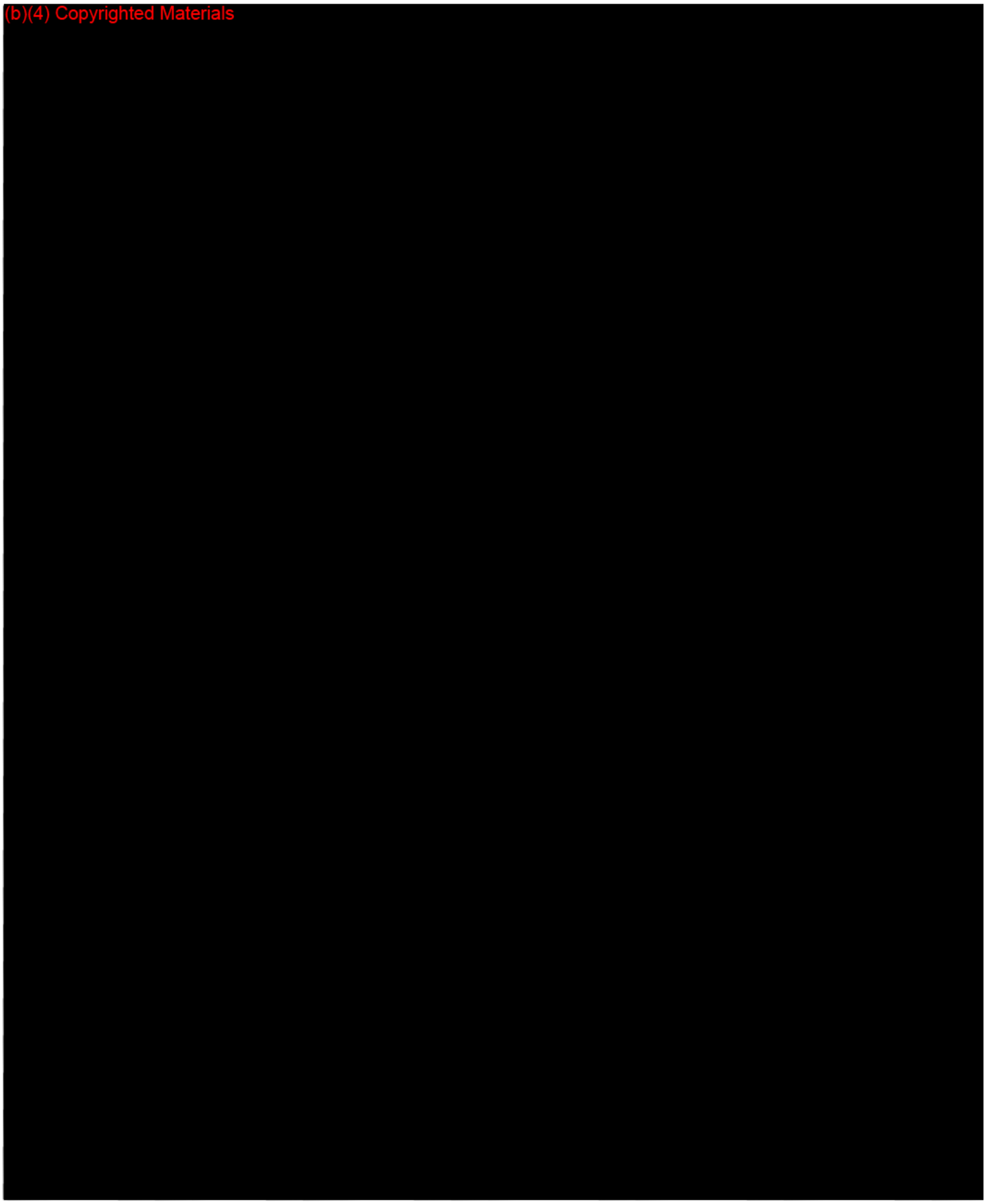

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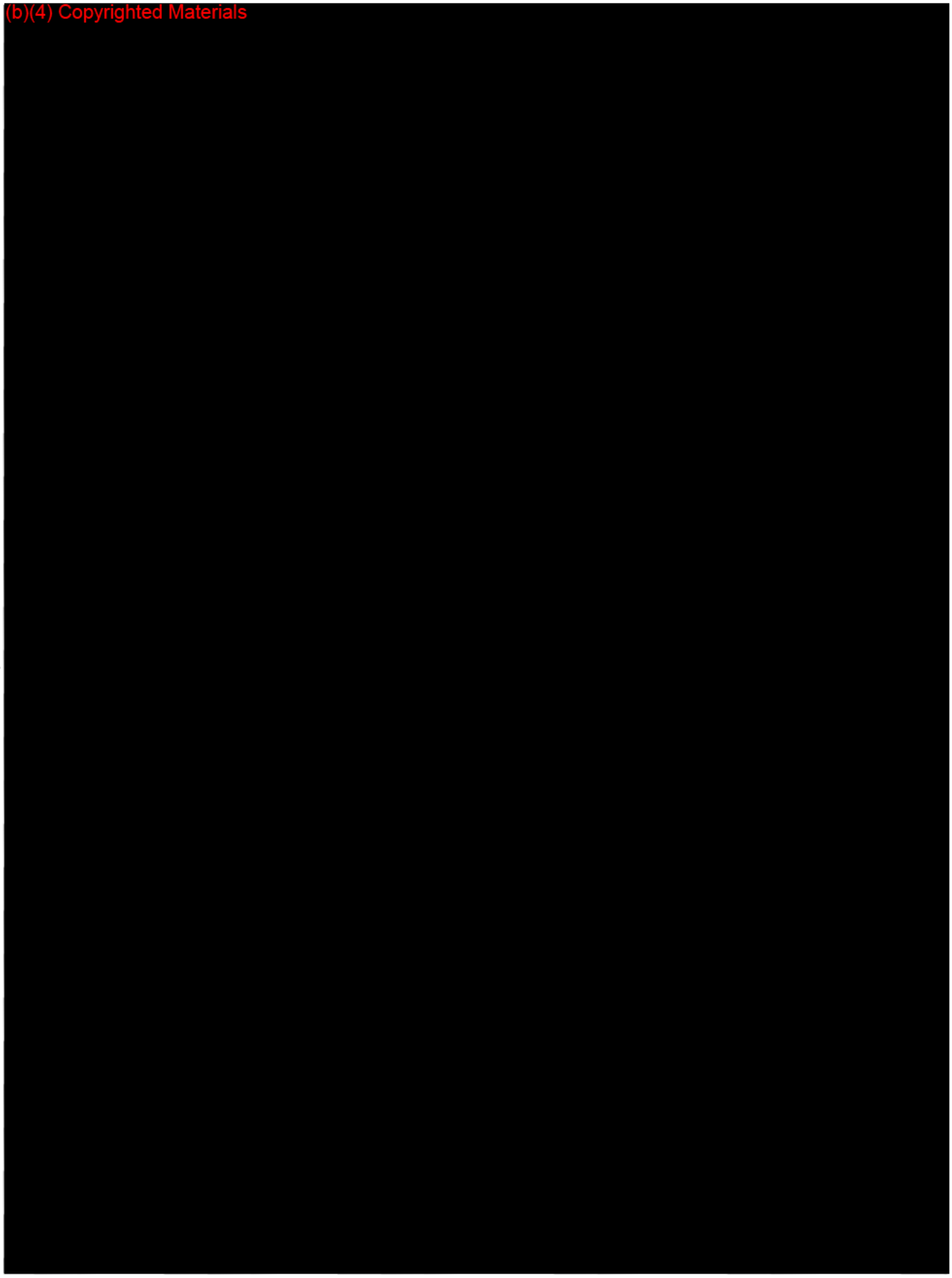

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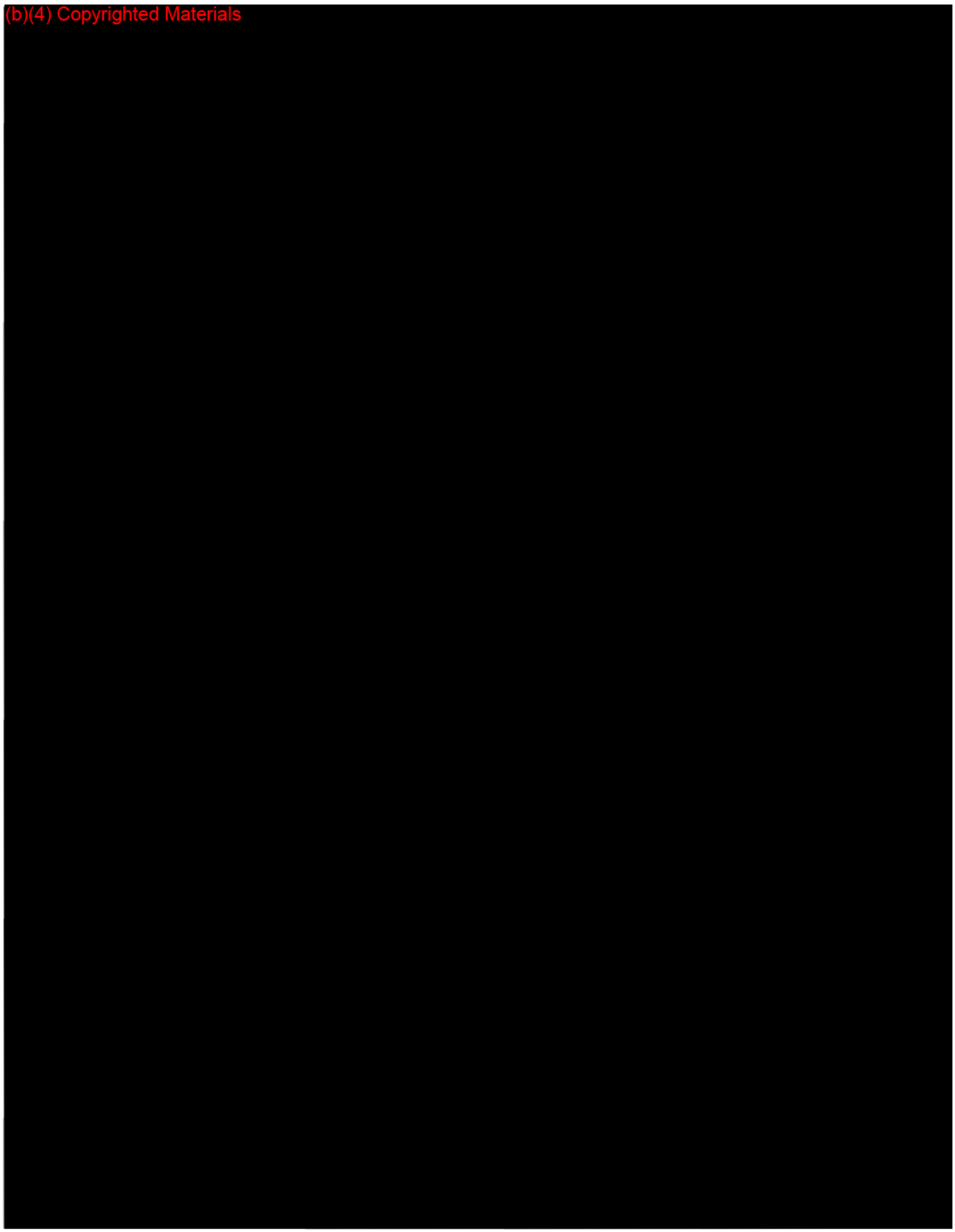

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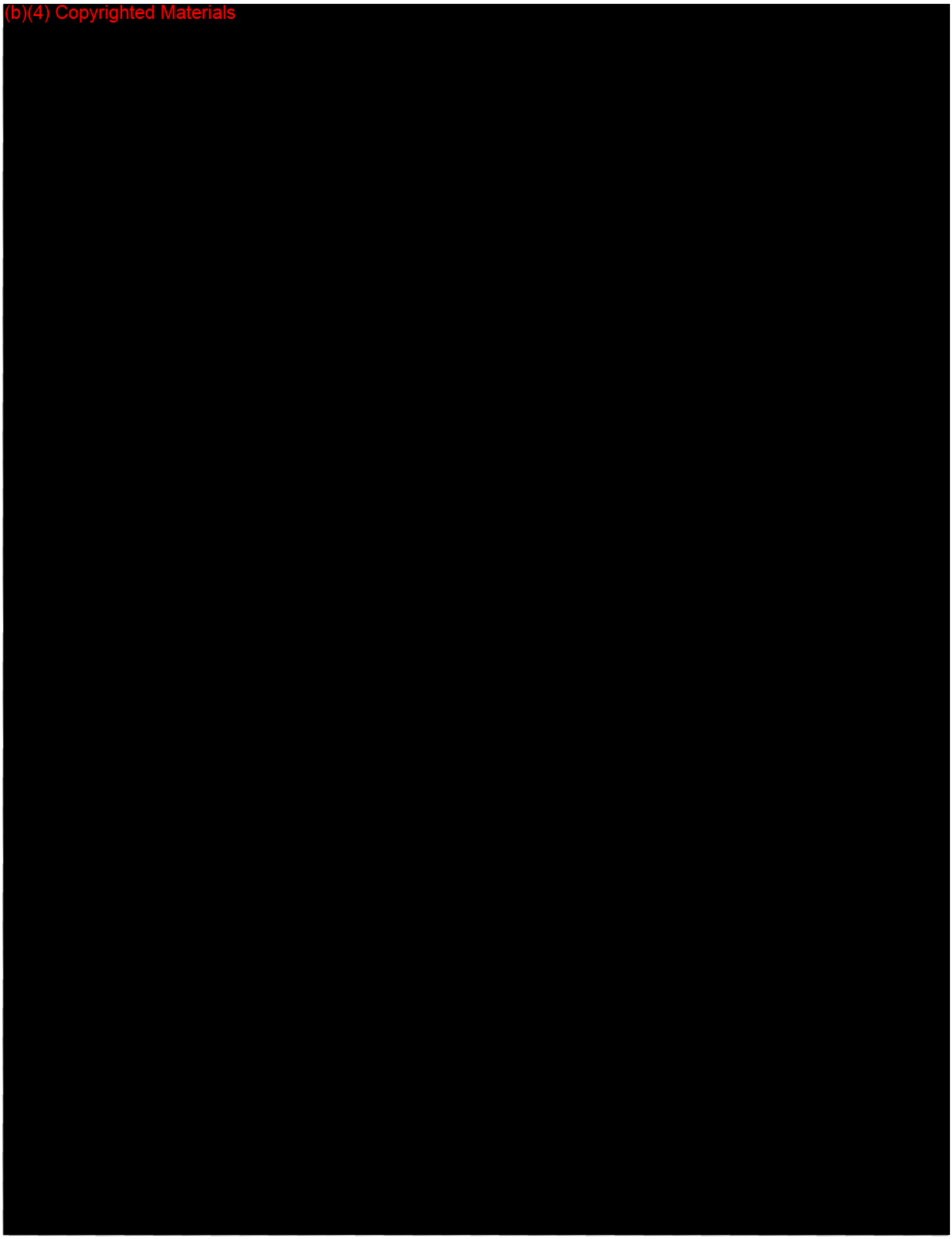

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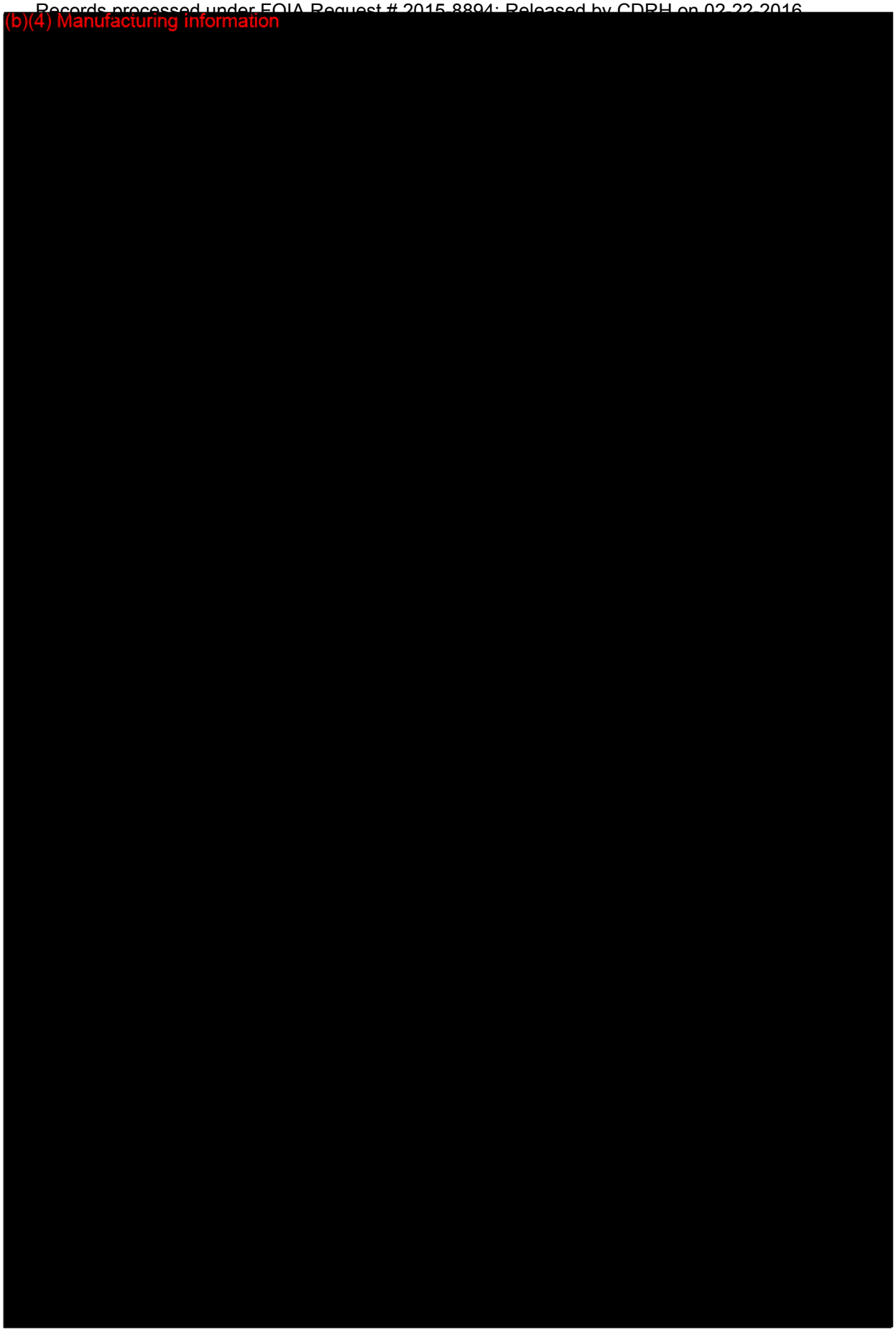

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Methotrexate Injection, USP

℞ only

WARNINGS

METHOTREXATE SHOULD BE USED ONLY BY PHYSICIANS WHOSE KNOWLEDGE AND EXPERIENCE INCLUDE THE USE OF ANTIMETABOLITE THERAPY.

BECAUSE OF THE POSSIBILITY OF SERIOUS TOXIC REACTIONS (WHICH CAN BE FATAL):

METHOTREXATE SHOULD BE USED ONLY IN LIFE THREATENING NEOPLASTIC DISEASES, OR IN PATIENTS WITH PSORIASIS OR RHEUMATOID ARTHRITIS WITH SEVERE, RECALCITRANT, DISABLING DISEASE WHICH IS NOT ADEQUATELY RESPONSIVE TO OTHER FORMS OF THERAPY.

DEATHS HAVE BEEN REPORTED WITH THE USE OF METHOTREXATE IN THE TREATMENT OF MALIGNANCY, PSORIASIS, AND RHEUMATOID ARTHRITIS.

PATIENTS SHOULD BE CLOSELY MONITORED FOR BONE MARROW, LIVER, LUNG AND KIDNEY TOXICITIES. (See **PRECAUTIONS**).

PATIENTS SHOULD BE INFORMED BY THEIR PHYSICIAN OF THE RISKS INVOLVED AND BE UNDER A PHYSICIAN'S CARE THROUGHOUT THERAPY.

THE USE OF METHOTREXATE HIGH DOSE REGIMENS RECOMMENDED FOR OSTEOSARCOMA REQUIRES METICULOUS CARE. (See **DOSAGE AND ADMINISTRATION**.) HIGH DOSE REGIMENS FOR OTHER NEOPLASTIC DISEASES ARE INVESTIGATIONAL AND A THERAPEUTIC ADVANTAGE HAS NOT BEEN ESTABLISHED.

METHOTREXATE FORMULATIONS AND DILUENTS CONTAINING PRESERVATIVES MUST NOT BE USED FOR INTRATHECAL OR HIGH DOSE METHOTREXATE THERAPY.

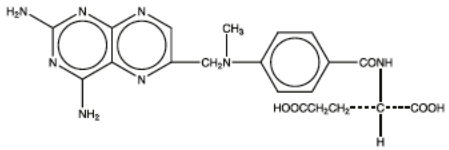
1. Methotrexate has been reported to cause fetal death and/or congenital anomalies.Therefore, it is not recommended for women of childbearing potential unless there is clear medical evidence that the benefits can be expected to outweigh the considered risks. Pregnant women with psoriasis or rheumatoid arthritis should not receive methotrexate. (See **CONTRAINDICATIONS**).
2. Methotrexate elimination is reduced in patients with impaired renal functions, ascites, or pleural effusions. Such patients require especially careful monitoring for toxicity, and require dose reduction or, in some cases, discontinuation of methotrexate administration.
3. Unexpectedly severe (sometimes fatal) bone marrow suppression, aplastic anemia, and gastrointestinal toxicity have been reported with concomitant administration of methotrexate (usually in high dosage) along with some nonsteroidal anti-inflammatory drugs (NSAIDs). (See **PRECAUTIONS**, **Drug Interactions**).
4. Methotrexate causes hepatotoxicity, fibrosis and cirrhosis, but generally only after prolonged use. Acutely, liver enzyme elevations are frequently seen. These are usually transient and asymptomatic, and also do not appear predictive of subsequent hepatic disease. Liver biopsy after sustained use often shows histologic changes, and fibrosis and cirrhosis have been reported; these latter lesions may not be preceded by symptoms or abnormal liver function tests in the psoriasis population. For this reason, periodic liver biopsies are usually recommended for psoriatic patients who are under long-term treatment. Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in the rheumatoid arthritis population. (See **PRECAUTIONS**, **Organ System Toxicity**, *Hepatic*).
5. Methotrexate-induced lung disease, including acute or chronic interstitial pneumonitis, is a potentially dangerous lesion, which may occur acutely at any time during therapy and has been reported at low doses. It is not always fully reversible and fatalities have been reported. Pulmonary symptoms (especially a dry, nonproductive cough) may require interruption of treatment and careful investigation.
6. Diarrhea and ulcerative stomatitis require interruption of therapy: otherwise, hemorrhagic enteritis and death from intestinal perforation may occur.
7. Malignant lymphomas, which may regress following withdrawal of methotrexate, may occur in patients receiving low-dose methotrexate and, thus, may not require cytotoxic treatment. Discontinue methotrexate first and, if the lymphoma does not regress, appropriate treatment should be instituted.
8. Like other cytotoxic drugs, methotrexate may induce "tumor lysis syndrome" in patients with rapidly growing tumors. Appropriate supportive and pharmacologic measures may prevent or alleviate this complication.
9. Severe, occasionally fatal, skin reactions have been reported following single or multiple doses of methotrexate. Reactions have occurred within days of oral, intramuscular, intravenous, or intrathecal methotrexate administration. Recovery has been reported with discontinuation of therapy. (See **PRECAUTIONS, Organ System Toxicity,** *Skin.*)
10. Potentially fatal opportunistic infections, especially *Pneumocystis carinii* pneumonia, may occur with methotrexate therapy.
11. Methotrexate given concomitantly with radiotherapy may increase the risk of soft tissue necrosis and osteonecrosis.

DESCRIPTION

Methotrexate (formerly Amethopterin) is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.

Chemically methotrexate is *N*-[4-[[(2,4-diamino-6-pteridinyl) methyl]methylamino]benzoyl]-L-glutamic acid.

The structural formula is:



Molecular weight: 454.45 $C_{20}H_{22}N_8O_5$

Methotrexate Injection, USP is sterile and non-pyrogenic and may be given by the intramuscular, intravenous or intra-arterial route. (See **DOSAGE AND ADMINISTRATION**). ***However, the preserved formulation contains Benzyl Alcohol and must not be used for intrathecal or high dose therapy.***

Methotrexate Injection, USP Isotonic Liquid, Contains Preservative is available in 25 mg/mL, 2 mL (50 mg) vials.

Each 25 mg/mL, 2 mL vial contains methotrexate sodium equivalent to 50 mg methotrexate, 0.9% w/v of Benzyl Alcohol as a preservative, and the following inactive ingredients: Sodium Chloride 0.260% w/v and Water for Injection qs ad 100% v. Sodium Hydroxide and, if necessary, Hydrochloric Acid are added to adjust the pH to approximately 8.5.

Methotrexate Injection, USP, Isotonic Liquid, Preservative Free, for single use only, is available in 10 mg/mL, 2 mL (20 mg) vials and 25 mg/mL, 20 mL (500 mg), 40 mL (1 g) and 100 mL (2.5 g) vials.

Each 10 mg/mL, 2 mL vial contains methotrexate sodium equivalent to 20 mg methotrexate, and the following inactive ingredients: Sodium Chloride 0.70% w/v. Sodium Hydroxide and, if necessary, Hydrochloric Acid are added to adjust the pH to approximately 8.5.

Each 25 mg/mL, 20 mL, 40 mL and 100 mL vial contains methotrexate sodium equivalent to 500 mg, 1 g and 2.5 g methotrexate, respectively, and the following inactive ingredients: Sodium Chloride 0.490% w/v. Sodium Hydroxide and, if necessary, Hydrochloric Acid are added to adjust the pH to approximately 8.5.

CLINICAL PHARMACOLOGY

Methotrexate inhibits dihydrofolic acid reductase. Dihydrofolates must be reduced to tetrahydrofolates by this enzyme before they can be utilized as carriers of one-carbon groups in the synthesis of purine nucleotides and thymidylate. Therefore, methotrexate interferes with DNA synthesis, repair, and cellular replication. Actively proliferating tissues such as malignant cells, bone marrow, fetal cells, buccal and intestinal mucosa, and cells of the urinary bladder are in general more sensitive to this effect of methotrexate. When cellular proliferation in malignant tissues is greater than in most normal tissues, methotrexate may impair malignant growth without irreversible damage to normal tissues.

The mechanism of action in rheumatoid arthritis is unknown; it may affect immune function. Two reports describe *in vitro* methotrexate inhibition of DNA precursor uptake by stimulated mononuclear cells, and another describes in animal polyarthritis partial correction by methotrexate of spleen cell hyporesponsiveness and suppressed IL 2 production. Other laboratories, however, have been unable to demonstrate similar effects. Clarification of methotrexate's effect on immune activity and its relation to rheumatoid immunopathogenesis await further studies.

In patients with rheumatoid arthritis, effects of methotrexate on articular swelling and tenderness can be seen as early as 3 to 6 weeks. Although methotrexate clearly ameliorates symptoms of inflammation (pain, swelling, stiffness), there is no evidence that it induces remission of rheumatoid arthritis nor has a beneficial effect been demonstrated on bone erosions and other radiologic changes which result in impaired joint use, functional disability, and deformity.

Most studies of methotrexate in patients with rheumatoid arthritis are relatively short term (3 to 6 months). Limited data from long-term studies indicate that an initial clinical improvement is maintained for at least two years with continued therapy.

In psoriasis, the rate of production of epithelial cells in the skin is greatly increased over normal skin. This differential in proliferation rates is the basis for the use of methotrexate to control the psoriatic process.

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Reference 483162-PROMOWEB

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Methotrexate in high doses, followed by leucovorin rescue, is used as a part of the treatment of patients with non-metastatic osteosarcoma. The original rationale for high dose methotrexate therapy was based on the concept of selective rescue of normal tissues by leucovorin. More recent evidence suggests that high dose methotrexate may also overcome methotrexate resistance caused by impaired active transport, decreased affinity of dihydrofolic acid reductase for methotrexate, increased levels of dihydrofolic acid reductase resulting from gene amplification, or decreased polyglutamation of methotrexate. The actual mechanism of action is unknown.

In a 6-month double-blind, placebo-controlled trial of 127 pediatric patients with juvenile rheumatoid arthritis (JRA) (mean age, 10.1 years; age range, 2.5 to 18 years; mean duration of disease, 5.1 years) on background nonsteroidal anti-inflammatory drugs (NSAIDs) and/or prednisone, methotrexate given weekly at an oral dose of 10 mg/m^2 provided significant clinical improvement compared to placebo as measured by either the physician's global assessment, or by a patient composite (25% reduction in the articular-severity score plus improvement in parent and physician global assessments of disease activity). Over two-thirds of the patients in this trial had polyarticular-course JRA, and the numerically greatest response was seen in this subgroup treated with 10 mg/m^2/wk methotrexate. The overwhelming majority of the remaining patients had systemic-course JRA. All patients were unresponsive to NSAIDs; approximately one-third were using low dose corticosteroids. Weekly methotrexate at a dose of 5 mg/m^2 was not significantly more effective than placebo in this trial.

Two Pediatric Oncology Group studies (one randomized and one non-randomized) demonstrated a significant improvement in relapse-free survival in patients with nonmetastatic osteosarcoma, when high dose methotrexate with leucovorin rescue was used in combination with other chemotherapeutic agents following surgical resection of the primary tumor. These studies were not designed to demonstrate the specific contribution of high dose methotrexate/leucovorin rescue therapy to the efficacy of the combination. However, a contribution can be inferred from the reports of objective responses to this therapy in patients with metastatic osteosarcoma, and from reports of extensive tumor necrosis following preoperative administration of this therapy to patients with non-metastatic osteosarcoma.

Pharmacokinetics

Absorption- In adults, oral absorption appears to be dose dependent. Peak serum levels are reached within one to two hours. At doses of 30 mg/m^2 or less, methotrexate is generally well absorbed with a mean bioavailability of about 60%. The absorption of doses greater than 80 mg/m^2 is significantly less, possibly due to a saturation effect.

In leukemic pediatric patients, oral absorption of methotrexate also appears to be dose dependent and has been reported to vary widely (23% to 95%). A twenty fold difference between highest and lowest peak levels (C_{max}: 0.11 to 2.3 micromolar after a 20 mg/m^2 dose) has been reported. Significant interindividual variability has also been noted in time to peak concentration (T_{max}: 0.67 to 4 hrs after a 15 mg/m^2 dose) and fraction of dose absorbed. The absorption of doses greater than 40 mg/m^2 has been reported to be significantly less than that of lower doses. Food has been shown to delay absorption and reduce peak concentration. Methotrexate is generally completely absorbed from parenteral routes of injection. After intramuscular injection, peak serum concentrations occur in 30 to 60 minutes. As in leukemic pediatric patients, a wide interindividual variability in the plasma concentrations of methotrexate has been reported in pediatric patients with JRA. Following oral administration of methotrexate in doses of 6.4 to 11.2 mg/m^2/week in pediatric patients with JRA, mean serum concentrations were 0.59 micromolar (range, 0.03 to 1.40) at 1 hour, 0.44 micromolar (range, 0.01 to 1.00) at 2 hours, and 0.29 micromolar (range, 0.06 to 0.58) at 3 hours. In pediatric patients receiving methotrexate for acute lymphocytic leukemia (6.3 to 30 mg/m^2), or for JRA (3.75 to 26.2 mg/m^2), the terminal half-life has been reported to range from 0.7 to 5.8 hours or 0.9 to 2.3 hours, respectively.

Distribution- After intravenous administration, the initial volume of distribution is approximately 0.18 L/kg (18% of body weight) and steady-state volume of distribution is approximately 0.4 to 0.8 L/kg (40 to 80% of body weight). Methotrexate competes with reduced folates for active transport across cell membranes by means of a single carrier-mediated active transport process. At serum concentrations greater than 100 micromolar, passive diffusion becomes a major pathway by which effective intracellular concentrations can be achieved. Methotrexate in serum is approximately 50% protein bound. Laboratory studies demonstrate that it may be displaced from plasma albumin by various compounds including sulfonamides, salicylates, tetracyclines, chloramphenicol, and phenytoin.

Methotrexate does not penetrate the blood-cerebrospinal fluid barrier in therapeutic amounts when given orally or parenterally. High CSF concentrations of the drug may be attained by intrathecal administration.

In dogs, synovial fluid concentrations after oral dosing were higher in inflamed than uninflamed joints. Although salicylates did not interfere with this penetration, prior prednisone treatment reduced penetration into inflamed joints to the level of normal joints.

Metabolism- After absorption, methotrexate undergoes hepatic and intracellular metabolism to polyglutamated forms which can be converted back to methotrexate by hydrolase enzymes. These polyglutamates act as inhibitors of dihydrofolate reductase and thymidylate synthetase. Small amounts of methotrexate polyglutamates may remain in tissues for extended periods. The retention and prolonged drug action of these active metabolites vary among different cells, tissues and tumors. A small amount of metabolism to 7-hydroxymethotrexate may occur at doses commonly prescribed. Accumulation of this metabolite may become significant at the high doses used in osteogenic sarcoma. The aqueous solubility of 7-hydroxymethotrexate is 3 to 5 fold lower than the parent compound. Methotrexate is partially metabolized by intestinal flora after oral administration.

Half-Life - The terminal half-life reported for methotrexate is approximately three to ten hours for patients receiving treatment for psoriasis, or rheumatoid arthritis or low dose antineoplastic therapy (less than 30 mg/m^2). For patients receiving high doses of methotrexate, the terminal half-life is eight to 15 hours.

Excretion - Renal excretion is the primary route of elimination and is dependent upon dosage and route of administration. With IV administration, 80% to 90% of the administered dose is excreted unchanged in the urine within 24 hours. There is limited biliary excretion amounting to 10% or less of the administered dose. Enterohepatic recirculation of methotrexate has been proposed.

Renal excretion occurs by glomerular filtration and active tubular secretion. Nonlinear elimination due to saturation of renal tubular reabsorption has been observed in psoriatic patients at doses between 7.5 and 30 mg. Impaired renal function, as well as concurrent use of drugs such as weak organic acids that also undergo tubular secretion, can markedly increase methotrexate serum levels. Excellent correlation has been reported between methotrexate clearance and endogenous creatinine clearance.

Methotrexate clearance rates vary widely and are generally at higher doses. Delayed drug clearance has been identified as one of the major factors responsible for methotrexate toxicity. It has been postulated that the toxicity of methotrexate for normal tissues is more dependent upon the duration of exposure to the drug rather than the peak level achieved. When a patient has delayed drug elimination due to compromised renal function, a third space effusion, or other causes, methotrexate serum concentrations may remain elevated for prolonged periods.

The potential for toxicity from high dose regimens or delayed excretion is reduced by the administration of leucovorin calcium during the final phase of methotrexate plasma elimination.

Pharmacokinetic monitoring of methotrexate serum concentrations may help identify those patients at high risk for methotrexate toxicity and aid in proper adjustments of leucovorin dosing. Guidelines for monitoring serum methotrexate levels, and for adjustment of leucovorin dosing to reduce the risk of methotrexate toxicity, are provided below in **DOSAGE AND ADMINISTRATION**.

Methotrexate has been detected in human breast milk. The highest breast milk to plasma concentration ratio reached was 0.08:1.

INDICATIONS AND USAGE

Neoplastic Diseases

Methotrexate is indicated in the treatment of gestational choriocarcinoma, chorioadenoma destruens and hydatidiform mole.

In acute lymphocytic leukemia, methotrexate is indicated in the prophylaxis of meningeal leukemia and is used in maintenance therapy in combination with other chemotherapeutic agents. Methotrexate is also indicated in the treatment of meningeal leukemia.

Methotrexate is used alone or in combination with other anticancer agents in the treatment of breast cancer, epidermoid cancers of the head and neck, advanced mycosis fungoides (cutaneous T cell lymphoma), and lung cancer, particularly squamous cell and small cell types. Methotrexate is also used in combination with other chemotherapeutic agents in the treatment of advanced stage non-Hodgkin's lymphomas.

Methotrexate in high doses followed by leucovorin rescue in combination with other chemotherapeutic agents is effective in prolonging relapse-free survival in patients with non-metastatic osteosarcoma who have undergone surgical resection or amputation for the primary tumor.

Psoriasis

Methotrexate is indicated in the symptomatic control of severe, recalcitrant, disabling psoriasis that is not adequately responsive to other forms of therapy, *but only when the diagnosis has been established, as by biopsy and/or after dermatologic consultation.* It is important to ensure that a psoriasis "flare" is not due to an undiagnosed concomitant disease affecting immune responses.

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Rheumatoid Arthritis including Polyarticular-Course Juvenile Rheumatoid Arthritis

Methotrexate is indicated in the management of selected adults with severe, active rheumatoid arthritis (ACR criteria), or children with active polyarticular-course juvenile rheumatoid arthritis, who have had an insufficient therapeutic response to, or are intolerant of, an adequate trial of first-line therapy including full dose non-steroidal anti-inflammatory agents (NSAIDs).

Aspirin, (NSAIDs), and/or low dose steroids may be continued, although the possibility of increased toxicity with concomitant use of NSAIDs including salicylates has not been fully explored. (See **PRECAUTIONS, Drug Interactions**.) Steroids may be reduced gradually in patients who respond to methotrexate. Combined use of methotrexate with gold, penicillamine, hydroxychloroquine, sulfasalazine, or cytotoxic agents, has not been studied and may increase the incidence of adverse effects. Rest and physiotherapy as indicated should be continued.

CONTRAINDICATIONS

Methotrexate can cause fetal death or teratogenic effects when administered to a pregnant woman. Methotrexate is contraindicated in pregnant women with psoriasis or rheumatoid arthritis and should be used in the treatment of neoplastic diseases only when the potential benefit outweighs the risk to the fetus. Women of childbearing potential should not be started on methotrexate until pregnancy is excluded and should be fully counseled on the serious risk to the fetus (see **PRECAUTIONS**) should they become pregnant while undergoing treatment. Pregnancy should be avoided if either partner is receiving methotrexate; during and for a minimum of three months after therapy for male patients, and during and for at least one ovulatory cycle after therapy for female patients. (See Boxed **WARNINGS**).

Because of the potential for serious adverse reactions from methotrexate in breast fed infants, it is contraindicated in nursing mothers.

Patients with psoriasis or rheumatoid arthritis with alcoholism, alcoholic liver disease or other chronic liver disease should not receive methotrexate.

Patients with psoriasis or rheumatoid arthritis who have overt or laboratory evidence of immunodeficiency syndromes should not receive methotrexate.

Patients with psoriasis or rheumatoid arthritis who have preexisting blood dyscrasias, such as bone marrow hypoplasia, leukopenia, thrombocytopenia, or significant anemia, should not receive methotrexate.

Patients with a known hypersensitivity to methotrexate should not receive the drug.

WARNINGS - SEE BOXED WARNINGS.

Methotrexate formulations and diluents containing preservatives must not be used for intrathecal or high dose methotrexate therapy.

PRECAUTIONS

General

Methotrexate has the potential for serious toxicity (See Boxed **WARNINGS**). Toxic effects may be related in frequency and severity to dose or frequency of administration but have been seen at all doses. Because they can occur at any time during therapy, it is necessary to follow patients on methotrexate closely. Most adverse reactions are reversible if detected early. When such reactions do occur, the drug should be reduced in dosage or discontinued and appropriate corrective measures should be taken. If necessary, this could include the use of leucovorin calcium and/or acute, intermittent hemodialysis with a high-flux dialyzer. (See **OVERDOSAGE**). If methotrexate therapy is reinstituted, it should be carried out with caution, with adequate consideration of further need for the drug and increased alertness as to possible recurrence of toxicity.

The clinical pharmacology of methotrexate has not been well studied in older individuals. Due to diminished hepatic and renal function as well as decreased folate stores in this population, relatively low doses should be considered, and these patients should be closely monitored for early signs of toxicity.

Some of the effects mentioned under **ADVERSE REACTIONS**, such as dizziness and fatigue, may affect the ability to drive or operate machinery.

Information for Patients

Patients should be informed of the early signs and symptoms of toxicity, of the need to see their physician promptly if they occur, and the need for close follow-up, including periodic laboratory tests to monitor toxicity.

Both the physician and pharmacist should emphasize to the patient that the recommended dose is taken weekly in rheumatoid arthritis and psoriasis, and that mistaken daily use of the recommended dose has led to fatal toxicity. Prescriptions should not be written or refilled on a PRN basis.

Patients should be informed of the potential benefit and risk in the use of methotrexate. The risk of effects on reproduction should be discussed with both male and female patients taking methotrexate.

Laboratory Tests

Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Baseline assessment should include a complete blood count with differential and platelet counts, hepatic enzymes, renal function tests and a chest X-ray. During therapy of rheumatoid arthritis and psoriasis, monitoring of these parameters is recommended: hematology at least monthly, renal function and liver function every 1 to 2 months. More frequent monitoring is usually indicated during antineoplastic therapy. *During initial or changing doses*, or during periods of increased risk of elevated methotrexate blood levels (e.g., dehydration), more frequent monitoring may also be indicated.

Transient liver function test abnormalities are observed frequently after methotrexate administration and are usually not cause for modification of methotrexate therapy. Persistent liver function test abnormalities, and/or depression of serum albumin may be indicators of serious liver toxicity and require evaluation. (See **PRECAUTIONS, Organ System Toxicity,** *Hepatic*).

A relationship between abnormal liver function tests and fibrosis or cirrhosis of the liver has not been established for patients with psoriasis. Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in the rheumatoid arthritis population.

Pulmonary function tests may be useful if methotrexate-induced lung disease is suspected, especially if baseline measurements are available.

Drug Interactions

Nonsteroidal anti-inflammatory drugs should not be administered prior to or concomitantly with the high doses of methotrexate, such as used in the treatment of osteosarcoma. Concomitant administration of some NSAIDs with high dose methotrexate therapy has been reported to elevate and prolong serum methotrexate levels, resulting in deaths from severe hematologic and gastrointestinal toxicity.

Caution should be used when NSAIDs and salicylates are administered concomitantly with lower doses of methotrexate. These drugs have been reported to reduce the tubular secretion of methotrexate in an animal model and may enhance its toxicity.

Despite the potential interactions, studies of methotrexate in patients with rheumatoid arthritis have usually included concurrent use of constant dosage regimens of NSAIDs, without apparent problems. It should be appreciated, however, that the doses used in rheumatoid arthritis (7.5 to 15 mg/week) are somewhat lower than those used in psoriasis and that larger doses could lead to unexpected toxicity.

Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored.

In the treatment of patients with osteosarcoma, caution must be exercised if high-dose methotrexate is administered in combination with a potentially nephrotoxic chemotherapeutic agent (e.g., cisplatin).

Methotrexate increases the plasma levels of mercaptopurine. The combination of methotrexate and mercaptopurine may therefore require dose adjustment.

Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria.

Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

The potential for increased hepatotoxicity when methotrexate is administered with other hepatotoxic agents has not been evaluated. However, hepatotoxicity has been reported in such cases. Therefore, patients receiving concomitant therapy with methotrexate and other potential hepatotoxins (e.g., azathioprine, retinoids, sulfasalazine) should be closely monitored for possible increased risk of hepatotoxicity.

Methotrexate may decrease the clearance of theophylline; theophylline levels should be monitored when used concurrently with methotrexate.

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Reference 483162-PROMOWEB

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Vitamin preparations containing folic acid or its derivatives may decrease responses to systemically administered methotrexate. Preliminary animal and human studies have shown that small quantities of intravenously administered leucovorin enter the CSF primarily as 5-methyltetrahydrofolate and, in humans, remain 1 to 3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration. However, high doses of leucovorin may reduce the efficacy of intrathecally administered methotrexate.

Folate deficiency states may increase methotrexate toxicity. Trimethoprim/sulfamethoxazole has been reported rarely to increase bone marrow suppression in patients receiving methotrexate, probably by decreased tubular secretion and/or an additive antifolate effect.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No controlled human data exist regarding the risk of neoplasia with methotrexate. Methotrexate has been evaluated in a number of animal studies for carcinogenic potential with inconclusive results. Although there is evidence that methotrexate causes chromosomal damage to animal somatic cells and human bone marrow cells, the clinical significance remains uncertain. Non-Hodgkin's lymphoma and other tumors have been reported in patients receiving low-dose oral methotrexate. However, there have been instances of malignant lymphoma arising during treatment with low-dose oral methotrexate, which have regressed completely following withdrawal of methotrexate, without requiring active anti-lymphoma treatment. Benefits should be weighed against the potential risk before using methotrexate alone or in combination with other drugs, especially in pediatric patients or young adults. Methotrexate causes embryotoxicity, abortion, and fetal defects in humans. It has also been reported to cause impairment of fertility, oligospermia and menstrual dysfunction in humans, during and for a short period after cessation of therapy.

Pregnancy

Psoriasis and rheumatoid arthritis: Methotrexate is in Pregnancy Category X. See **CONTRAINDICATIONS**.

Nursing Mothers

See **CONTRAINDICATIONS**.

Pediatric Use

Safety and effectiveness in pediatric patients have been established only in cancer chemotherapy and in polyarticular-course juvenile rheumatoid arthritis.

Published clinical studies evaluating the use of methotrexate in children and adolescents (i.e., patients 2 to 16 years of age) with JRA demonstrated safety comparable to that observed in adults with rheumatoid arthritis (see **CLINICAL PHARMACOLOGY, ADVERSE REACTIONS** and **DOSAGE AND ADMINISTRATION**.)

Methotrexate injectable formulations containing the preservative benzyl alcohol are not recommended for use in neonates. There have been reports of fatal 'gasping syndrome' in neonates (children less than one month of age) following the administrations of intravenous solutions containing the preservative benzyl alcohol. Symptoms include a striking onset of gasping respiration, hypotension, bradycardia, and cardiovascular collapse.

Serious neurotoxicity, frequently manifested as generalized or focal seizures, has been reported with unexpectedly increased frequency among pediatric patients with acute lymphoblastic leukemia who were treated with intermediate-dose intravenous methotrexate (1 gm/m^2). (See **PRECAUTIONS, Organ System Toxicity**, *Neurologic*).

Geriatric Use

Clinical studies of methotrexate did not include sufficient numbers of subjects age 65 and over to determine whether they respond differently from younger subjects. In general, dose selection for an elderly patient should be cautious reflecting the greater frequency of decreased hepatic and renal function, decreased folate stores, concomitant disease or other drug therapy (i.e., that interfere with renal function, methotrexate or folate metabolism) in this population (See **PRECAUTIONS, Drug Interactions**). Since decline in renal function may be associated with increases in adverse events and serum creatinine measurements may over estimate renal function in the elderly, more accurate methods (i.e., creatinine clearance) should be considered. Serum methotrexate levels may also be helpful. Elderly patients should be closely monitored for early signs of hepatic, bone marrow and renal toxicity. In chronic use situations, certain toxicities may be reduced by folate supplementation. Post-marketing experience suggests that the occurrence of bone marrow suppression, thrombocytopenia, and pneumonitis may increase with age. See Boxed **WARNINGS** and **ADVERSE REACTIONS**.

Organ System Toxicity

Gastrointestinal: If vomiting, diarrhea, or stomatitis occur, which may result in dehydration, methotrexate should be discontinued until recovery occurs. Methotrexate should be used with extreme caution in the presence of peptic ulcer disease or ulcerative colitis.

Hematologic: Methotrexate can suppress hematopoiesis and cause anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, and/or thrombocytopenia. In patients with malignancy and preexisting hematopoietic impairment, the drug should be used with caution, if at all. In controlled clinical trials in rheumatoid arthritis (n=128), leukopenia (WBC <3000/mm^3) was seen in 2 patients, thrombocytopenia (platelets <100,000/mm^3) in 6 patients, and pancytopenia in 2 patients.

In psoriasis and rheumatoid arthritis, methotrexate should be stopped immediately if there is a significant drop in blood counts. In the treatment of neoplastic diseases, methotrexate should be continued only if the potential benefit warrants the risk of severe myelosuppression. Patients with profound granulocytopenia and fever should be evaluated immediately and usually require parenteral broad-spectrum antibiotic therapy.

Hepatic: Methotrexate has the potential for acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatotoxicity. Chronic toxicity is potentially fatal; it generally has occurred after prolonged use (generally two years or more) and after a total dose of at least 1.5 grams. In studies in psoriatic patients, hepatotoxicity appeared to be a function of total cumulative dose and appeared to be enhanced by alcoholism, obesity, diabetes and advanced age. An accurate incidence rate has not been determined; the rate of progression and reversibility of lesions is not known. Special caution is indicated in the presence of preexisting liver damage or impaired hepatic function.

In psoriasis, liver function tests, including serum albumin, should be performed periodically prior to dosing but are often normal in the face of developing fibrosis or cirrhosis. These lesions may be detectable only by biopsy. The usual recommendation is to obtain a liver biopsy at 1) pretherapy or shortly after initiation of therapy (2 to 4 months), 2) a total cumulative dose of 1.5 grams, and 3) after each additional 1.0 to 1.5 grams. Moderate fibrosis or any cirrhosis normally leads to discontinuation of the drug; mild fibrosis normally suggests a repeat biopsy in 6 months. Milder histologic findings such as fatty change and low grade portal inflammation, are relatively common pretherapy. Although these mild changes are usually not a reason to avoid or discontinue methotrexate therapy, the drug should be used with caution.

In rheumatoid arthritis, age at first use of methotrexate and duration of therapy have been reported as risk factors for hepatotoxicity; other risk factors, similar to those observed in psoriasis, may be present in rheumatoid arthritis but have not been confirmed to date. Persistent abnormalities in liver function tests may precede appearance of fibrosis or cirrhosis in this population. There is a combined reported experience in 217 rheumatoid arthritis patients with liver biopsies both before and during treatment (after a cumulative dose of at lease 1.5 g) and in 714 patients with a biopsy only during treatment. There are 64 (7%) cases of fibrosis and 1 (0.1%) case of cirrhosis. Of the 64 cases of fibrosis, 60 were deemed mild. The reticulin stain is more sensitive for early fibrosis and its use may increase these figures. It is unknown whether even longer use will increase these risks.

Liver function tests should be performed at baseline at 4 to 8 week intervals in patients receiving methotrexate for rheumatoid arthritis. Pretreatment liver biopsy should be performed for patients with a history of excessive alcohol consumption, persistently abnormal baseline liver function test values or chronic hepatitis B or C infection. During therapy, liver biopsy should be performed if there are persistent liver function test abnormalities or there is a decrease in serum albumin below the normal range (in the setting of well controlled rheumatoid arthritis).

If the results of a liver biopsy show mild changes (Roenigk, grades I, II, IIIa), methotrexate may be continued and the patient monitored as per recommendations listed above. Methotrexate should be discontinued in any patient who displays persistently abnormal liver function tests and refuses liver biopsy or in any patient whose liver biopsy shows moderate to severe changes (Roenigk grade IIIb or IV).

Infection or Immunologic States: Methotrexate should be used with extreme caution in the presence of active infection, and is usually contraindicated in patients with overt or laboratory evidence of immunodeficiency syndromes. Immunization may be ineffective when given during methotrexate therapy. Immunization with live virus vaccines is generally not recommended. There have been reports of disseminated vaccinia infections after smallpox immunizations in patients receiving methotrexate therapy. Hypogammaglobulinemia has been reported rarely.

Potentially fatal opportunistic infections, especially *Pneumocystis carinii* pneumonia, may occur with methotrexate therapy. When a patient presents with pulmonary symptoms, the possibility of *Pneumocystis carinii* pneumonia should be considered.

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Neurologic: There have been reports of leukoencephalopathy following intravenous administration of methotrexate to patients who have had craniospinal irradiation. Serious neurotoxicity, frequently manifested as generalized or focal seizures, has been reported with unexpectedly increased frequency among pediatric patients with acute lymphoblastic leukemia who were treated with intermediate-dose intravenous methotrexate (1 gm/m²). Symptomatic patients were commonly noted to have leukoencephalopathy and/or microangiopathic calcifications on diagnostic imaging studies. Chronic leukoencephalopathy has also been reported in patients who received repeated doses of high-dose methotrexate with leucovorin rescue even without cranial irradiation. Discontinuation of methotrexate does not always result in complete recovery.

A transient acute neurologic syndrome has been observed in patients treated with high dose regimens. Manifestations of this stroke-like encephalopathy may include confusion, hemiparesis, transient blindness, seizures and coma. The exact cause is unknown.

After the intrathecal use of methotrexate, the central nervous system toxicity which may occur can be classified as follows: acute chemical arachnoiditis manifested by such symptoms as headache, back pain, nuchal rigidity, and fever; sub-acute myelopathy characterized by paraparesis/paraplegia associated with involvement with one or more spinal nerve roots; chronic leukoencephalopathy manifested by confusion, irritability, somnolence, ataxia, dementia, seizures and coma. This condition can be progressive and even fatal.

Pulmonary: Pulmonary symptoms (especially a dry nonproductive cough) or a non-specific pneumonitis occurring during methotrexate therapy may be indicative of a potentially dangerous lesion and require interruption of treatment and careful investigation. Although clinically variable, the typical patient with methotrexate induced lung disease presents with fever, cough, dyspnea, hypoxemia, and an infiltrate on chest X-ray; infection (including pneumonia) needs to be excluded. This lesion can occur at all dosages.

Renal: Methotrexate may cause renal damage that may lead to acute renal failure. High doses of methotrexate used in the treatment of osteosarcoma may cause renal damage leading to acute renal failure. Nephrotoxicity is due primarily to the precipitation of methotrexate and 7-hydroxymethotrexate in the renal tubules. Close attention to renal function including adequate hydration, urine alkalinization and measurement of serum methotrexate and creatinine levels are essential for safe administration.

Skin: Severe, occasionally fatal, dermatologic reactions, including toxic epidermal necrolysis, Stevens-Johnson syndrome, exfoliative dermatitis, skin necrosis, and erythema multiforme, have been reported in children and adults, within days of oral, intramuscular, intravenous, or intrathecal methotrexate administration. Reactions were noted after single or multiple low, intermediate, or high doses of methotrexate in patients with neoplastic and non-neoplastic diseases.

Other precautions: Methotrexate should be used with extreme caution in the presence of debility.

Methotrexate exits slowly from third space compartments (e.g., pleural effusions or ascites). This results in a prolonged terminal plasma half-life and unexpected toxicity. In patients with significant third space accumulations, it is advisable to evacuate the fluid before treatment and to monitor plasma methotrexate levels.

Lesions of psoriasis may be aggravated by concomitant exposure to ultraviolet radiation. Radiation dermatitis and sunburn may be "recalled" by the use of methotrexate.

ADVERSE REACTIONS

IN GENERAL, THE INCIDENCE AND SEVERITY OF ACUTE SIDE EFFECTS ARE RELATED TO DOSE AND FREQUENCY OF ADMINISTRATION. THE MOST SERIOUS REACTIONS ARE DISCUSSED ABOVE UNDER ORGAN SYSTEM TOXICITY IN THE PRECAUTION SECTION. THAT SECTION SHOULD ALSO BE CONSULTED WHEN LOOKING FOR INFORMATION ABOUT ADVERSE REACTIONS WITH METHOTREXATE.

The most frequently reported adverse reactions include ulcerative stomatitis, leukopenia, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, undue fatigue, chills and fever, dizziness and decreased resistance to infection.

Other adverse reactions that have been reported with methotrexate are listed below by organ system. In the oncology setting, concomitant treatment and the underlying disease make specific attribution of a reaction to methotrexate difficult.

Alimentary System: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis, pancreatitis.

Blood and Lymphatic System Disorders: suppressed hematopoiesis, anemia, aplastic anemia, pancytopenia, leukopenia, neutropenia, thrombocytopenia, agranulocytosis, eosinophilia, lymphadenopathy and lymphoproliferative disorders (including reversible). Hypogammaglobulinemia has been reported rarely.

Cardiovascular: pericarditis, pericardial effusion, hypotension, and thromboembolic events (including arterial thrombosis, cerebral thrombosis, deep vein thrombosis, retinal vein thrombosis, thrombophlebitis, and pulmonary embolus).

Central Nervous System: headaches, drowsiness, blurred vision, transient blindness, speech impairment including dysarthria and aphasia, hemiparesis, paresis and convulsions have also occurred following administration of methotrexate. Following low doses, there have been occasional reports of transient subtle cognitive dysfunction, mood alteration or unusual cranial sensations, leukoencephalopathy, or encephalopathy.

Hepatobiliary Disorders: hepatotoxicity, acute hepatitis, chronic fibrosis and cirrhosis, hepatic failure, decrease in serum albumin, liver enzyme elevations.

Infection: There have been case reports of sometimes fatal opportunistic infections in patients receiving methotrexate therapy for neoplastic and non-neoplastic diseases. *Pneumocystis carinii* pneumonia was the most common opportunistic infection. There have also been reports of infections, pneumonia, Cytomegalovirus infection, including cytomegaloviral pneumonia, sepsis, fatal sepsis, nocardiosis; histoplasmosis, cryptococcosis, *Herpes zoster, H. simplex* hepatitis, and disseminated *H. simplex.*

Musculoskeletal System: stress fracture.

Ophthalmic: conjunctivitis, serious visual changes of unknown etiology.

Pulmonary System: respiratory fibrosis, respiratory failure, alveolitis, interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred.

Skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis, erythema multiforme, toxic epidermal necrolysis, Stevens-Johnson syndrome, skin necrosis, skin ulceration and exfoliative dermatitis.

Urogenital System: severe nephropathy or renal failure, azotemia, cystitis, hematuria, proteinuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction, vaginal discharge, and gynecomastia; infertility, abortion, fetal death, fetal defects.

Other rarer reactions related to or attributed to the use of methotrexate such as nodulosis, vasculitis, arthralgia/myalgia, loss of libido/impotence, diabetes, osteoporosis, sudden death, lymphoma, including reversible lymphomas, tumor lysis syndrome, soft tissue necrosis and osteonecrosis. Anaphylactoid reactions have been reported.

Adverse Reactions in Double-Blind Rheumatoid Arthritis Studies

The approximate incidences of methotrexate-attributed (i.e. placebo rate subtracted) adverse reactions in 12 to 18 week double-blind studies of patients (n=128) with rheumatoid arthritis treated with low-dose oral (7.5 to 15 mg/week) pulse methotrexate, are listed below. Virtually all of these patients were on concomitant nonsteroidal anti-inflammatory drugs and some were also taking low dosages of corticosteroids. Hepatic histology was not examined in these short-term studies. (See **PRECAUTIONS**).

Incidence greater than 10%: Elevated liver function tests 15%, nausea/vomiting 10%.

Incidence 3% to 10%: Stomatitis, thrombocytopenia (platelet count less than 100,000/mm³).

Incidence 1% to 3%: Rash/pruritis/dermatitis, diarrhea, alopecia, leukopenia (WBC less than 3000/mm³), pancytopenia, dizziness.

Two other controlled trials of patients (n=680) with Rheumatoid Arthritis on 7.5 mg to 15 mg/wk oral doses showed an incidence of interstitial pneumonitis of 1%. (See **PRECAUTIONS**.)

Other less common reactions included decreased hematocrit, headache, upper respiratory infection, anorexia, arthralgias, chest pain, coughing, dysuria, eye discomfort, epistatix, fever, infection, sweating, tinnitus, and vaginal discharge.

Adverse Reactions in Psoriasis:

There are no recent placebo-controlled trials in patients with psoriasis. There are two literature reports (Roenigk, 1969, and Nyfors, 1978) describing large series (n=204, 248) of psoriasis patients treated with methotrexate. Dosages ranged up to 25 mg per week and treatment was administered for up to four years. With the exception of alopecia, photosensitivity, and "burning of skin lesions" (each 3% to 10%), the adverse reaction rates in these reports were very similar to those in the rheumatoid arthritis studies. Rarely, painful plaque erosions may appear (Pearce, HP and Wilson, BB: *Am Acad Dermatol* 35: 835-838, 1996).

Adverse Reactions in JRA Studies

The approximate incidences of adverse reactions reported in pediatric patients with JRA treated with oral, weekly doses of methotrexate (5 to 20 mg/m²/wk or 0.1 to 0.65 mg/kg/wk) were as follows (virtually all patients were receiving concomitant nosteroidal anti-inflammatory drugs, and some also were taking low doses of corticosteroids): elevated liver function tests, 14%; gastrointestinal reactions (e.g., nausea, vomiting, diarrhea), 11%; stomatitis, 2%; leukopenia, 2%; headache, 1.2%; alopecia, 0.5%; dizziness, 0.2%; and rash, 0.2%. Although there is experience with dosing up to 30 mg/m²/wk in JRA, the published data for doses above 20 mg/m²/wk are too limited to provide reliable estimates of adverse reaction rates.

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OVERDOSAGE

Leucovorin is indicated to diminish the toxicity and counteract the effect of inadvertently administered overdosages of methotrexate. Leucovorin administration should begin as promptly as possible. As the time interval between methotrexate administration and leucovorin initiation increases, the effectiveness of leucovorin in counteracting toxicity decreases. Monitoring of the serum methotrexate concentration is essential in determining the optimal dose and duration of treatment with leucovorin.

In cases of massive overdosage, hydration and urinary alkalinization may be necessary to prevent the precipitation of methotrexate and/or its metabolites in the renal tubules. Generally speaking, neither hemodialysis nor peritoneal dialysis has been shown to improve methotrexate elimination. However, effective clearance of methotrexate has been reported with acute, intermittent hemodialysis using a high-flux dialyzer (Wall, SM et al: *Am J Kidney Dis* 28 (6): 846-854, 1996).

Accidental intrathecal overdosage may require intensive systemic support, high-dose systemic leucovorin, alkaline diuresis and rapid CSF drainage and ventriculolumbar perfusion.

In postmarketing experience, overdose with methotrexate has generally occurred with oral and intrathecal administration, although intravenous and intramuscular overdose have also been reported.

Reports of oral overdose often indicate accidental daily administration instead of weekly (single or divided doses). Symptoms commonly reported following oral overdose include those symptoms and signs reported at pharmacologic doses, particularly hematologic and gastrointestinal reaction. For example, leukopenia, thrombocytopenia, anemia, pancytopenia, bone marrow suppression, mucositis, stomatitis, oral ulceration, nausea, vomiting, gastrointestinal ulceration, gastrointestinal bleeding. In some cases, no symptoms were reported. There have been reports of death following overdose. In these cases, events such as sepsis or septic shock, renal failure, and aplastic anemia were also reported.

Symptoms of intrathecal overdose are generally central nervous system (CNS) symptoms, including headache, nausea and vomiting, seizure or convulsion, and acute toxic encephalopathy. In some cases, no symptoms were reported. There have been reports of death following intrathecal overdose. In these cases, cerebellar herniation associated with increased intracranial pressure, and acute toxic encephalopathy have also been reported.

There are published case reports of intravenous and intrathecal carboxypeptidase G2 treatment to hasten clearance of methotrexate in cases of overdose.

DOSAGE AND ADMINISTRATION

Neoplastic Diseases

Oral administration in tablet form is often preferred when low doses are being administered since absorption is rapid and effective serum levels are obtained. Methotrexate injection may be given by the intramuscular, intravenous or intra-arterial route. ***However, the preserved formulation contains Benzyl Alcohol and must not be used for intrathecal or high dose therapy.*** Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Choriocarcinoma and similar trophoblastic diseases: Methotrexate is administered orally or intramuscularly in doses of 15 to 30 mg daily for a five-day course. Such courses are usually repeated for 3 to 5 times as required, with rest periods of one or more weeks interposed between courses, until any manifesting toxic symptoms subside. The effectiveness of therapy is ordinarily evaluated by 24 hour quantitative analysis of urinary chorionic gonadotropin (hCG), which should return to normal or less than 50 IU/24 hr usually after the third or fourth course and usually be followed by a complete resolution of measurable lesions in 4 to 6 weeks. One to two courses of methotrexate after normalization of hCG is usually recommended. Before each course of the drug careful clinical assessment is essential. Cyclic combination therapy of methotrexate with other antitumor drugs has been reported as being useful.

Since hydatidiform mole may precede choriocarcinoma, prophylactic chemotherapy with methotrexate has been recommended.

Chorioadenoma destruens is considered to be an invasive form of hydatidiform mole.

Methotrexate is administered in these disease states in doses similar to those recommended for choriocarcinoma.

Leukemia: Acute lymphoblastic leukemia in pediatric patients and young adolescents is the most responsive to present day chemotherapy. In young adults and older patients, clinical remission is more difficult to obtain and early relapse is more common.

Methotrexate alone or in combination with steroids was used initially for induction of remission in acute lymphoblastic leukemias. More recently corticosteroid therapy, in combination with other antileukemic drugs or in cyclic combinations with methotrexate included, has appeared to produce rapid and effective remissions.

When used for induction, methotrexate in doses of 3.3 mg/m² in combination with 60 mg/m² of prednisone, given daily, produced remissions in 50% of patients treated, usually within a period of 4 to 6 weeks. Methotrexate in combination with other agents appears to be the drug of choice for securing maintenance of drug-induced remissions. When remission is achieved and supportive care has produced general clinical improvement, maintenance therapy is initiated, as follows: Methotrexate is administered 2 times weekly either by mouth or intramuscularly in total weekly doses of 30 mg/m². It has also been given in doses of 2.5 mg/kg intravenously every 14 days. If and when relapse does occur, reinduction of remission can again usually be obtained by repeating the initial induction regimen.

A variety of combination chemotherapy regimens have been used for both induction and maintenance therapy in acute lymphoblastic leukemia. The physician should be familiar with the new advances in antileukemic therapy.

Meningeal Leukemia: In the treatment of prophylaxis of meningeal leukemia, methotrexate must be administered intrathecally. Preservative free methotrexate is diluted to a concentration of 1 mg/mL in an appropriate sterile, preservative free medium such as 0.9% Sodium Chloride Injection, USP.

The cerebrospinal fluid volume is dependent on age and not on body surface area. The CSF is at 40% of the adult volume at birth and reaches the adult volume in several years.

Intrathecal methotrexate administration at a dose of 12 mg/m² (maximum 15 mg) has been reported to result in low CSF methotrexate concentrations and reduced efficacy in pediatric patients and high concentrations and neurotoxicity in adults. The following dosage regimen is based on age instead of body surface area:

AGE (years)	DOSE (mg)
<1	6
1	8
2	10
3 or older	12

In one study in patients under the age of 40, this dosage regimen appeared to result in more consistent CSF methotrexate concentrations and less neurotoxicity. Another study in pediatric patients with acute lymphocytic leukemia compared this regimen to a dose of 12 mg/m² (maximum 15 mg), a significant reduction in the rate of CNS relapse was observed in the group whose dose was based on age.

Because the CSF volume and turnover may decrease with age, a dose reduction may be indicated in elderly patients.

For treatment of meningeal leukemia, intrathecal methotrexate may be given at intervals of 2 to 5 days. However, administration at intervals of less than 1 week may result in increased subacute toxicity. Methotrexate is administered until the cell count of the cerebrospinal fluid returns to normal. At this point one additional dose is advisable. For prophylaxis against meningeal leukemia, the dosage is the same as for treatment except for the intervals of administration. On this subject, it is advisable for the physician to consult the medical literature.

Untoward side effects may occur with any given intrathecal injection and are commonly neurological in character. Large doses may cause convulsions. Methotrexate given by the intrathecal route appears significantly in the systemic circulation and may cause systemic methotrexate toxicity. Therefore, systemic antileukemic therapy with the drug should be appropriately adjusted, reduced or discontinued. Focal leukemic involvement of the central nervous system may not respond to intrathecal chemotherapy and is best treated with radiotherapy.

Lymphomas: In Burkitt's tumor, Stages I-II, methotrexate has produced prolonged remissions in some cases. Recommended dosage is 10 to 25 mg/day orally for 4 to 8 days. In Stage III, methotrexate is commonly given concomitantly with other antitumor agents. Treatment in all stages usually consists of several courses of the drug interposed with 7 to 10 day rest periods. Lymphosarcomas in Stage III may respond to combined drug therapy with methotrexate given in doses of 0.625 to 2.5 mg/kg daily.

Mycosis fungoides (cutaneous T cell lymphoma): Therapy with methotrexate as a single agent appears to produce clinical responses in up to 50% of patients treated. Dosage in early stages is usually 5 to 50 mg once weekly. Dose reduction or cessation is guided by patient response and hematologic monitoring. Methotrexate has also been administered twice weekly in doses ranging from 15 to 37.5 mg in patients who have responded poorly to weekly therapy. Combination chemotherapy regimens that include intravenous methotrexate administered at higher doses with leucovorin rescue have been utilized in advanced stages of the disease.

Osteosarcoma: An effective adjuvant chemotherapy regimen requires the administration of several cytotoxic chemotherapeutic agents. In addition to high-dose methotrexate with leucovorin rescue, these agents may

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include doxorubicin, cisplatin, and the combination of bleomycin, cyclophosphamide and dactinomycin (BCD) in the doses and schedule shown in the table below. The starting dose for high-dose methotrexate treatment is 12 grams/m^2. If this dose is not sufficient to produce a peak serum methotrexate concentration of 1,000 micromolar (10^{-3} mol/L) at the end of the methotrexate infusion, the dose may be escalated to 15 grams/m^2 in subsequent treatments. If the patient is vomiting or is unable to tolerate oral medication, leucovorin is given IV or IM at the same dose and schedule.

Drug*	Dose*	Treatment Week After Surgery
Methotrexate	12 g/m^2 IV as 4 hour infusion (starting dose)	4,5,6,7,11,12,15,16,29,30,44,45
Leucovorin	15 mg orally every six hours for 10 doses starting at 24 hours after start of methotrexate infusion	- - -
Doxorubicin† as a single drug	30 mg/m^2 day IV x 3 days	8,17
Doxorubicin†	50 mg/m^2 IV	20,23,33,36
Cisplatin†	100 mg/m^2 IV	20,23,33,36
Bleomycin†	15 units/m^2 IV x 2 days	2,13,26,39,42
Cyclophosphamide†	600 mg/m^2 IV x 2 days	2,13,26,39,42
Dactinomycin†	0.6 mg/m^2 IV x 2 days	2,13,26,39,42

*Link MP, Goorin AM, Miser AW, et al: The effect of adjuvant chemotherapy on relapse-free survival in patients with osteosarcoma of the extremity. *N Engl J of Med* 1986; 314 (No.25): 1600-1606.
†See each respective package insert for full prescribing information. Dosage modifications may be necessary because of drug-induced toxicity.

When these higher doses of methotrexate are to be administered, the following safety guidelines should be closely observed.

GUIDELINES FOR METHOTREXATE THERAPY WITH LEUCOVORIN RESCUE

1. Administration of methotrexate should be delayed until recovery if:
   - the WBC count is less than 1500/microliter
   - the neutrophil count is less than 200/microliter
   - the platelet count is less than 75,000/microliter
   - the serum bilirubin level is greater than 1.2 mg/dL
   - the SGPT level is greater than 450 U
   - mucositis is present, until there is evidence of healing
   - persistent pleural effusion is present; this should be drained dry prior to infusion.
2. Adequate renal function must be documented.
   a. Serum creatinine must be normal, and creatinine clearance must be greater than 60 mL/min, before initiation of therapy.
   b. Serum creatinine must be measured prior to each subsequent course of therapy. If serum creatinine has increased by 50% or more compared to a prior value, the creatinine clearance must be measured and documented to be greater than 60 mL/min (even if the serum creatinine is still within the normal range).
3. Patients must be well hydrated, and must be treated with sodium bicarbonate for urinary alkalinization.
   a. Administer 1,000 mL/m^2 of intravenous fluid over 6 hours prior to initiation of the methotrexate infusion. Continue hydration at 125 mL/m^2/hr (3 liters/m^2/day) during the methotrexate infusion, and for 2 days after the infusion has been completed.
   b. Alkalinize urine to maintain pH above 7.0 during methotrexate infusion and leucovorin calcium therapy. This can be accomplished by the administration of sodium bicarbonate orally or by incorporation into a separate intravenous solution.
4. Repeat serum creatinine and serum methotrexate 24 hours after starting methotrexate and at least once daily until the methotrexate level is below 5 x 10^{-8} mol/L (0.05 micromolar).
5. The table below provides guidelines for leucovorin calcium dosage based upon serum methotrexate levels. (See table below.‡)
   Patients who experience delayed early methotrexate elimination are likely to develop nonreversible oliguric renal failure. In addition to appropriate leucovorin therapy, these patients require continuing hydration and urinary alkalinization, and close monitoring of fluid and electrolyte status, until the serum methotrexate level has fallen to below 0.05 micromolar and the renal failure has resolved. If necessary, acute, intermittent hemodialysis with a high-flux dialyzer may also be beneficial in these patients.
6. Some patients will have abnormalities in methotrexate elimination, or abnormalities in renal function following methotrexate administration, which are significant but less severe than the abnormalities described in the table below. These abnormalities may or may not be associated with significant clinical toxicity. If significant toxicity is observed, leucovorin rescue should be extended for an additional 24 hours (total 14 doses over 84 hours) in subsequent courses of therapy. The possibility that the patient is taking other medications which interact with methotrexate (e.g., medications which may interfere with methotrexate binding to serum albumin, or elimination) should always be reconsidered when laboratory abnormalities or clinical toxicities are observed.

CAUTION: DO NOT ADMINISTER LEUCOVORIN INTRATHECALLY.

Psoriasis, Rheumatoid Arthritis, and Juvenile Rheumatoid Arthritis

Adult Rheumatoid Arthritis: Recommended Starting Dosage Schedules

1. Single oral doses of 7.5 mg once weekly.†
2. Divided oral dosages of 2.5 mg at 12 hour intervals for 3 doses given as a course once weekly.†

† Methotrexate Sodium Tablets for oral administration are available.

Polyarticular-Course Juvenile Rheumatoid Arthritis: The recommended starting dose is 10 mg/m^2 given once weekly.

For either adult RA or polyarticular-course JRA, dosages may be adjusted gradually to achieve an optimal response. Limited experience shows a significant increase in the incidence and severity of serious toxic reactions, especially bone marrow suppression, at doses greater than 20 mg/wk in adults. Although there is experience with doses up to 30 mg/m^2/wk in children, there are too few published data to assess how doses over 20 mg/m^2/wk might affect the risk of serious toxicity in children. Experience does suggest, however, that children receiving 20 to 30 mg/m^2/wk (0.65 to 1.0 mg/kg/wk) may have better absorption and fewer gastrointestinal side effects if methotrexate is administered either intramuscularly or subcutaneously.

Therapeutic response usually begins within 3 to 6 weeks and the patient may continue to improve for another 12 weeks or more.

The optimal duration of therapy is unknown. Limited data available from long-term studies in adults indicate that the initial clinical improvement is maintained for at least two years with continued therapy. When methotrexate is discontinued, the arthritis usually worsens within 3 to 6 weeks.

The patient should be fully informed of the risks involved and should be under constant supervision of the physician. (See **Information for Patients** under **PRECAUTIONS**). Assessment of hematologic, hepatic, renal, and pulmonary function should be made by history, physical examination, and laboratory tests before beginning, periodically during, and before reinstituting methotrexate therapy. (See **PRECAUTIONS**). Appropriate steps should be taken to avoid conception during methotrexate therapy. (See **PRECAUTIONS** and **CONTRAINDICATIONS**).

All schedules should be continually tailored to the individual patient. An initial test dose may be given prior to the regular dosing schedule to detect any extreme sensitivity to adverse effects (See **ADVERSE REACTIONS**). Maximal myelosuppression usually occurs in seven to ten days.

Psoriasis: Recommended Starting Dose Schedule:

1. Weekly single oral, IM or IV dosage schedule: 10 to 25 mg per week until adequate response is achieved.†
2. Divided oral dose schedule 2.5 mg at 12 hour intervals for three doses.†

†Methotrexate Sodium Tablets for oral administration are available.

Dosages in each schedule may be gradually adjusted to achieve optimal clinical response; 30 mg/week should not ordinarily be exceeded.

Once optimal clinical response has been achieved, each dosage schedule should be reduced to the lowest possible amount of drug and to the longest possible rest period. The use of methotrexate may permit the return to conventional topical therapy, which should be encouraged.

HANDLING AND DISPOSAL

Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-7] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

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DILUTION INSTRUCTIONS FOR LIQUID METHOTREXATE INJECTION PRODUCT

Methotrexate Injection, USP, Isotonic Liquid, Contains Preservative

If desired, the solution may be further diluted with a compatible medium such as Sodium Chloride Injection, USP. Storage for 24 hours at a temperature of 21°C to 25°C results in a product which is within 90% of label potency.

Methotrexate Injection, USP, Isotonic Liquid, Preservative Free, for Single Use Only

If desired, the solution may be further diluted immediately prior to use with an appropriate sterile, preservative free medium such as 5% Dextrose Solution, USP or Sodium Chloride Injection, USP.

HOW SUPPLIED

Parenteral:

Methotrexate Injection, USP, Isotonic Liquid, Contains Preservative. Each 25 mg/mL, 2 mL vial contains methotrexate sodium equivalent to 50 mg methotrexate.

50 mg, 2 mL Vial	NDC 61703-350-38

Methotrexate Injection, USP, Isotonic Liquid, Preservative Free, for Single Use Only. Each 10 mg/mL, 2 mL vial contains methotrexate sodium equivalent to 20 mg methotrexate.

20 mg, 2 mL Vial	NDC 61703-352-07

Methotrexate Injection, USP, Isotonic Liquid, Preservative Free, for Single Use Only. Each 25 mg/mL, 20 mL, 40 mL and 100 mL vial contains methotrexate sodium equivalent to 500 mg, 1 g and 2.5 g methotrexate respectively.

500 mg, 20 mL Vial	NDC 61703-408-22
1 g, 40 mL Vial	NDC 61703-408-41
2.5 g, 100 mL Vial	NDC 61703-351-59

Store at controlled room temperature, 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F). PROTECT FROM LIGHT.

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Revision August 2007

‡LEUCOVORIN RESCUE SCHEDULES FOLLOWING TREATMENT WITH HIGHER DOSES OF METHOTREXATE

Clinical Situation	Laboratory Findings	Leucovorin Dosage and Duration
Normal Methotrexate Elimination	Serum methotrexate level approximately 10 micromolar at 24 hours after administration, 1 micromolar at 48 hours, and less than 0.2 micromolar at 72 hours.	15 mg PO, IM, or IV q 6 hours for 60 hours (10 doses starting at 24 hours after start of methotrexate infusion).
Delayed Late Methotrexate Elimination	Serum methotrexate level remaining above 0.2 micromolar at 72 hours, and more than 0.05 micromolar at 96 hours after administration.	Continue 15 mg PO, IM, or IV q six hours, until methotrexate level is less than 0.05 micromolar.
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	Serum methotrexate level of 50 micromolar or more at 24 hours, or 5 micromolar or more at 48 hours after administration, OR; a 100% or greater increase in serum creatinine level at 24 hours after methotrexate administration, (e.g., an increase from 0.5 mg/dL to a level of 1 mg/dL or more).	150 mg IV q three hours, until methotrexate level is less than 1 micromolar; then 15 mg IV q three hours until methotrexate level is less than 0.05 micromolar.

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Indication for Use

510(K) Number (if known):

Device Name:

ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control
ARK™ Methotrexate Dilution Buffer

Indications for Use:

The ARK™ Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of Methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

The ARK™ Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay.

The ARK™ Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay.

The ARK™ Methotrexate Dilution Buffer is intended for the dilution of specimens containing high concentrations of methotrexate.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Prescription Use ___X___	And/Or	Over the Counter Use ____.
(21 CFR Part 801 Subpart D)		(21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

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7-Hydroxymethotrexate as a Urinary Metabolite in Human Subjects and Rhesus Monkeys Receiving High Dose Methotrexate

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ABSTRACT Human subjects and rhesus monkeys receiving the antitumor agent methotrexate at the high dose levels recently introduced into clinical use (> 50 mg/kg) excrete significant amounts of the metabolite 7-hydroxymethotrexate. The metabolite is not detected in these species after methotrexate therapy at conventional dose levels. The evidence indicates that in primates, the in vivo conversion of methotrexate to 7-hydroxymethotrexate is a dose-dependent phenomenon, with the enzyme system(s) catalyzing the reaction having a low affinity for the drug.

INTRODUCTION

The folate antagonist methotrexate (MTX; 4-amino-4-deoxy-N^{10}-methylpteroylglutamate) [1] is in general clinical use as a single agent or in combination with other antineoplastic agents in the treatment of choriocarcinoma, Burkitt's lymphoma, acute leukemia of childhood, epidermoid carcinoma of the head and neck, and breast cancer (1, 2). Recently, "high-dose" MTX therapy (> 50 mg MTX/kg) with leucovorin rescue has been shown to be of value in the treatment of osteogenic sarcoma (3, 4). The toxicity of this regimen is significant, with renal failure having been observed, in addition to the gastrointestinal ulceration and bone marrow depression seen with conventional MTX therapy. Earlier studies of the clinical pharmacology of MTX administered at conventional dose levels indicate that in man and other primates, the compound is not metabolized but is excreted unchanged in the urine (5–7). We now report evidence that at the high dose levels presently in clinical use, MTX undergoes quantitatively significant conversion both in man and the rhesus monkey to a metabolite with extremely limited aqueous solubility, 7-hydroxymethotrexate.

Received for publication 11 September 1975 and in revised form 17 November 1975.

[1] *Abbreviations used in this paper:* MTX, methotrexate; 7-hydroxyMTX, 7-hydroxymethotrexate.

METHODS

Five human male subjects with osteogenic sarcoma or with epidermoid carcinoma of the lung, ranging in age from 11 to 48 yr, were given high dose MTX (50–200 mg/kg) [2] in 6-h i.v. infusions. Three normal mature female rhesus monkeys were given either MTX or [3′,5′,9-^{3}H]MTX (8) (sp act 148 μCi/mmol) in 6-h i.v. infusions at a dose-level of 200 mg/kg. Beginning with the start of the infusion, urine was serially collected over time periods of 0–6, 6–12, 12–18, and 18–24 h for the human subjects, and 0–6, 6–12, and 12–24 h for the monkeys. 3-ml aliquots of the collected urines were adjusted to pH 8.3 and chromatographed on columns of DEAE-cellulose with ammonium bicarbonate gradient elution over a concentration range of 0.1–0.4 M (9). 100 6-ml fractions were collected. The position of elution of MTX in this chromatographic system (fractions 20–26) was determined from ultraviolet and visible absorption spectra of the individual fractions, by assay of the fractions for dihydrofolate reductase inhibitory activity (10), and in the experiments with tritium-labeled MTX, by liquid scintillation counting. Monkeys were killed 24 h after the start of the infusion and kidney tissue slices were prepared for histological and autoradiographic studies. Samples of the same kidneys were also stored at −8°C for later determination of MTX content by dihydrofolate reductase inhibition assay and by measurement of tritium levels.

[2] The purity of MTX used in the high dose clinical studies (Lederle Laboratories, Pearl River, N. Y.) was assessed by chromatography on DEAE-cellulose. The material was found to be homogeneous except for a small amount (< 3%) of the previously reported compound N^{10}-methylpteroylglutamate (7), a by-product of the manufacturing process.

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TABLE I

Urinary Excretion of 7-HydroxyMTX by Human Subjects in Early (6–12 h) and Late (18–24 h) Time Periods after the i.v. Administration of MTX in the High Dose Range (>50 mg/kg)

Patient	Age	Diagnosis	Dose	Total dose	6–12-h Collection period*		18–24-h Collection period*	
					MTX plus 7-OH-MTX excreted	Percentage as 7-OH-MTX	MTX plus 7-OH-MTX excreted	Percentage as 7-OH-MTX
	yr		*mg/kg*	*g*	*mg*	%	*mg*	%
R. F.	11	Osteogenic sarcoma	200	8.0	1,550	2.1	112	33
S. J.	29	Osteogenic sarcoma	100	9.3	1,830	4.0	152	30
F. K.	31	Osteogenic sarcoma	100	7.0	1,029	9.6	78	22
G. R.	48	Epidermoid cancer of lung	50	3.0	652	0.7	179	7
W. C.	35	Osteogenic sarcoma	50	2.8	1,348	0.9	9	10

All patients studied were male.

* The difference between percentage 7-OH-MTX excreted in the 6–12-h collection period and in the 18–24-h collection period is significant at $P < 0.05$, utilizing Student's two-tailed t test.

RESULTS

Of unusual interest in the chromatographic studies was the unexpected finding of a slowly migrating MTX-derived compound (fractions 59–69) which was present at detectable levels in the monkey urines after 6 h and in all the human urines examined (Table I). Typical elution patterns for this compound and for MTX in the human and monkey urines are shown in Fig. 1. As a percentage of total excreted drug, the excretion of the metabolite was greater in the later time periods, ranging from 7 to 33% of excreted drug in the 18–24-h human urine samples vs. only 0.7–9.6% in the 6–12-h samples (Table I). The slowly migrating metabolite was identical to authentic 7-hydroxymethotrexate (7-hydroxyMTX) in its chromatographic behavior on DEAE-cellulose, its ultraviolet and visible absorption spectra, its infrared absorption spectrum (Fig. 2), and its weak activity as an inhibitor of mammalian dihydrofolate reductase (11). The aqueous solubility of 7-hydroxyMTX was determined over the pH range 5.0–7.0 and found to be three- to fivefold less than that of MTX (Table II).

Histological sections and autoradiographs of tissue slices from the kidneys of monkeys which had received tritium-labeled MTX 24 h previously revealed crystalline-like deposits of MTX or MTX-derived material within the lumen of the renal tubules. The glomeruli appeared normal. The autoradiograph (Fig. 3) shows the crystalline-like deposits within the tubules, with exposed silver grains in the emulsion overlying the deposits. On DEAE-cellulose chromatography of the protein-free supernatant fractions from homogenates of the same kidney tissue used in the autoradiographic studies, it was found that only 23% of the tritium radioactivity in the kidney was present as MTX, while the remainder was present as 7-hydroxyMTX.

DISCUSSION

The consistent presence of 7-hydroxyMTX in urines of human subjects and rhesus monkeys receiving MTX, and in renal tissues from monkeys receiving MTX, was unexpected in view of previous reports from this and other laboratories (5–7), that MTX in these two species is excreted essentially unchanged. These early studies were carried out at conventional dose levels, while the present studies were carried out at dose levels some 5- to 2,000-fold higher in man and 600-fold higher in the monkey. From the early report by Henderson et al. (7), it is apparent from the chromatographic and spectrophotometric data cited that 7-hydroxyMTX was detected as a minor metabolite, although not identified, at the highest dose of MTX used by these workers in studies with human subjects (10 mg/kg); since the compound was not seen at lower dose levels, its presence was attributed to an artifact arising from bacterial metabolism of the drug after collection of the urine samples. That the metabolite is indeed generated in vivo, however, is clear from the present studies, in which 7-hydroxyMTX was detected in urine samples chromatographed immediately after

TABLE II

pH Dependence of Aqueous Solubility of 7-HydroxyMTX and MTX

Compound	Aqueous solubility, *mg/ml*		
	pH 5.0	pH 6.0	pH 7.0
7-HydroxyMTX	0.13	0.37	1.55
MTX	0.44	1.60	8.90

Concentrations of dissolved 7-hydroxyMTX and MTX were determined spectrophotometrically after a 2-h equilibration period in sodium phosphate buffer, 0.1 M, at 37°C.

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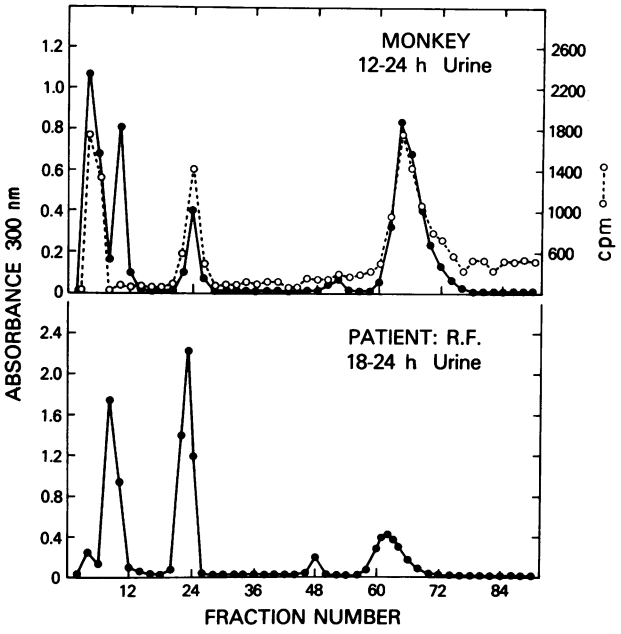

FIGURE 1 DEAE-cellulose chromatographic elution patterns of MTX and its metabolite in monkey and human urine after i.v. administration of MTX at a dose level of 200 mg/kg. (*Upper panel*) [3′,5′,9-^{3}H]MTX (Dhom Products, North Hollywood, Calif.; 10 Ci/mmol) was purified by column chromatography on DEAE-cellulose and diluted with unlabeled MTX to give a final sp act of 148 μCi/mmol. A female rhesus monkey (6 kg) was given 200 mg/kg (546 μCi) of the labeled drug in a 6-h i.v. infusion, and total urine collection was carried out over the time periods 0–6, 6–12, and 12–24 h after the start of the infusion. For the experiment recorded above, a 3-ml aliquot of the 12–24-h urine sample was applied to a DEAE-cellulose column (1.3 × 16 cm) and gradient elution carried out with ammonium bicarbonate over a concentration range of 0.1–0.4 M. 100 6-ml fractions were collected. 93% of ^{3}H radioactivity applied to the column was recovered. (●), Absorbance at 300 nm; (○), tritium radioactivity, cpm/0.2 ml eluate. The early peak of radioactivity (fractions 2–8) has been previously shown to be made up of cleavage products of MTX (7). The identities of the MTX and 7-hydroxyMTX peaks (fractions 20–26 and 59–69, respectively) were established from the site of elution of standard reference compounds, from the ultraviolet and visible absorption spectra of the peak fractions, and, in the case of MTX, from dihydrofolate reductase inhibition assay. The peak at fractions 46–54 is N^{10}-methylpteroylglutamate, a previously reported by-product of the manufacturing process for methotrexate (7). The quantity of MTX and 7-hydroxyMTX was determined by integration of the area under each peak. (*Lower panel*) A male patient, R. F. (40 kg), was given MTX, 200 mg/kg, in a 6-h i.v. infusion, and a 3-ml aliquot of the 18–24-h pooled urine chromatographed as described above. The results shown are from a single representative experiment; data from five patients are summarized in Table I.

collection, and in samples of renal tissue which were kept frozen until immediately before extracts were prepared for chromatography.

The unusual dose dependence for the appearance of 7-hydroxyMTX in the urine is most readily explained by postulating that the enzyme catalyzing the oxidation has only a low affinity for the parent drug. Other possibilities exist as well (e.g. saturation of an alternate route of excretion via the biliary tract), but are less

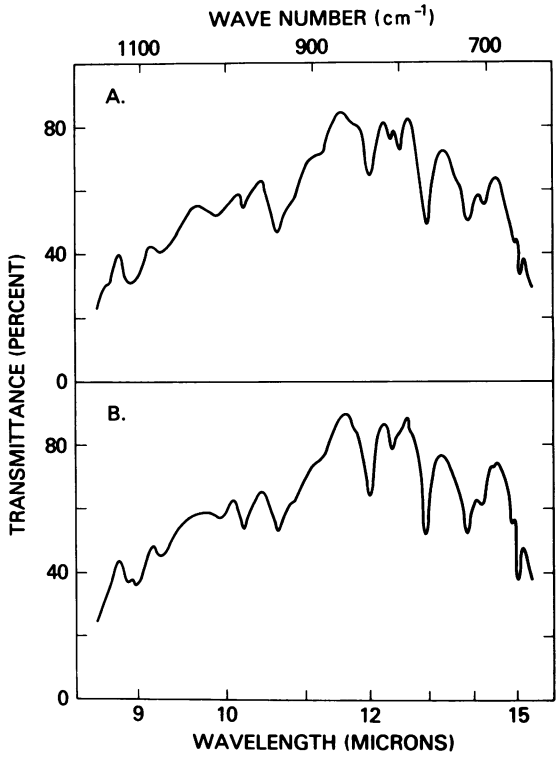

FIGURE 2 Infrared absorption spectra of (A) authentic 7-hydroxyMTX and (B) of the MTX metabolite isolated from monkey urine after the administration of MTX, 200 mg/kg. A female rhesus monkey (6 kg) was given MTX, 200 mg/kg in a 6-h i.v. infusion and the 12–24-h urine (110 ml) pooled and stored overnight at 5°C. The lemon-yellow precipitate which formed was collected by centrifugation and washed once with cold acetone. The precipitate was redissolved in sodium hydroxide solution, 0.05 N, and the insoluble residue discarded. Glacial acetic acid was added dropwise to the supernatant solution until a pH of 2.0 was reached. The solution was stored overnight at 5°C. The precipitate was washed twice with cold acetone and dried *in vacuo* over phosphorus pentoxide for 48 h. Yield was 80 mg. On chromatography on DEAE-cellulose, the material was found to be homogeneous and to migrate identically with authentic 7-hydroxyMTX. Spectra were recorded with a model 621 infrared spectrometer (Perkin-Elmer Corp., Norwalk, Conn.); compounds were suspended in a paraffin oil mull.

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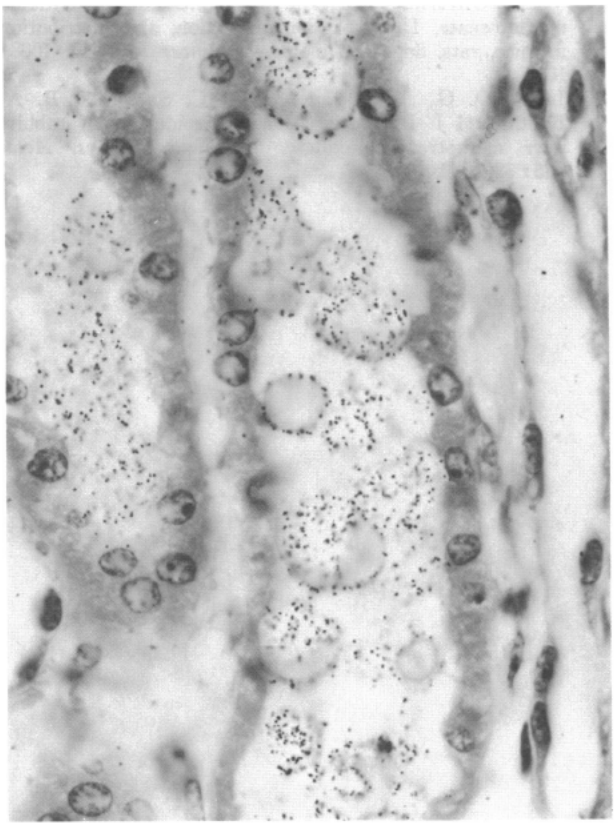

FIGURE 3 Kidney autoradiograph, × 860, from a rhesus monkey killed 24 h after administration of [3′,5′,9-^{3}H]MTX (546 μCi) at a dose of 200 mg/kg. The section is from the renal medulla. The tissue was immediately fixed in absolute alcohol. Tissue sections of 5 μm thickness were cut. The slides were dipped in Kodak Nuclear Track Emulsion NTB2 (Eastman Kodak Co., Rochester, N. Y.), heated to 45°C, exposed for 14 days, developed, and stained with hematoxylin and eosin.

likely since previous studies of biliary excretion of MTX administered at conventional dose levels in the monkey have not shown the presence of the 7-hydroxy metabolite (12). In studies of the 7-hydroxylation of MTX by the rabbit, in which species the reaction is quantitatively significant even at lower dose levels, the hydroxylation has been shown to be catalyzed by the hepatic metalloflavoprotein aldehyde oxidase (EC 1.2.-3.1); the Michaelis constant for the reaction with the rabbit enzyme is of the order of 0.1 mM (13). The latter concentration would be considerably exceeded in vivo after the administration of high-dose MTX (assuming equal distribution of the drug throughout body water), but would not be achieved after the administration of MTX in the conventional dose range. Whether hepatic aldehyde oxidase is responsible for the conversion in primates has not, however, been established, and is presently under investigation in this laboratory.

7-HydroxyMTX has previously been shown to be some two orders of magnitude less effective as an inhibitor of mammalian dihydrofolate reductase than is MTX (11). The metabolite would not therefore contribute significantly to the folate antagonist activity of MTX in vivo, and in this respect the 7-hydroxylation reaction can be considered a detoxication mechanism. In other respects, however, these findings are of possible clinical and toxicological significance. A frequent complication of high-dose MTX therapy is impairment of renal function, as evidenced by a rising serum creatinine level and a falling creatinine clearance (3). This syndrome can readily be reproduced in the monkey, and is associated in the latter species with the presence of deposits of MTX-derived crystalline material in the renal tubules (Fig. 3). Since both MTX and 7-hydroxy-MTX are limited in aqueous solubility, particularly at acid pH, both compounds may contribute to the formation of this crystalline material, although as noted above, the aqueous solubility of the 7-hydroxy metabolite is some three- to fivefold less than that of the parent compound.

ACKNOWLEDGMENTS

We thank Dr. Ti Li Loo for providing 7-hydroxyMTX prepared by chemical synthesis, Dr. John A. Beisler for the infrared absorption spectra, and Mr. C. Jerry Derr for skillful technical assistance.

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Eur J Cancer, Vol. 27, No. 8, pp. 1061–1062, 1991.
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Methotrexate Encephalopathy

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VOLUME 26 · NUMBER 27 · SEPTEMBER 20 2008

JOURNAL OF CLINICAL ONCOLOGY

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High-Dose Methotrexate in Osteosarcoma: Let the Questions Surcease—Time for Final Acceptance

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Osteosarcoma is the most common primary malignant bone tumor affecting children and adults. The etiology is unknown, although a higher incidence occurs in patients with bilateral retinoblastoma, Paget's disease of bone, the Li Fraumeni syndrome, and those who have received therapeutic radiation. The biologic behavior is consistent with the premise that silent pulmonary micrometastases are present in at least 80% of patients at diagnosis. These metastases are not visible on imaging studies, but in the absence of effective systemic therapy, they generally surface 6 to 9 months after surgical ablation of the primary tumor. If untreated, they will be responsible for the patients demise within 2 to 3 years of their appearance. Historical controls reveal a survival of 5% to 20%.

There are currently four major classes of chemotherapeutic agents with established efficacy that are used in the therapy of this malignancy: doxorubicin, cisplatin, ifosfamide/high-dose cyclophosphamide, and high-dose methotrexate with leucovorin rescue (MTX-L). Doxorubicin and MTX-L were discovered first in the early 1970s. Doxorubicin, and the other agents when discovered later, were accepted without query; not so for MTX-L. It was initially subjected to intense scrutiny,[1,2] rebuffed intermittently,[3] and later incorporated with variable degrees of indifference or enthusiasm into therapeutic regimens seemingly for want of a more effective agent. During this interminable period of probation (still extant[4]), support for its efficacy and confirmation of its activity emanated from various publications. It was shown to be effective in eliminating overt pulmonary metastases, improving the opportunity for limb salvage, healing pathologic fractures, and enhancing the effect of radiation therapy for palliation and control of inoperable tumors.[5] When administered postoperatively as the sole agent after ablation of the primary tumor, it increased survival to 40%,[6] and in combination with other agents (pre- and postoperative therapy), it increased survival to 65% to 75%.[6-12]

A regimen devoid of MTX (non–methotrexate-based therapy) has been documented, inter alia, among the "major poor prognostic factors."[10] The authors of this article stipulate that the major poor prognostic factors were as follows: "[age] older than 30 years, large absolute or relative tumor volume, tumor location in the proximal humerus, no neoadjuvant chemotherapy, non–methotrexate-based therapy, poor histologic response to preoperative chemotherapy. . . . . and so on."

Using serum MTX concentrations to monitor MTX levels, the regimen has been successfully administered safely and effectively to patients in numerous investigations.[6-12] MTX is usually administered over 4 to 6 hours, and it is generally considered that a mean peak serum MTX threshold greater than 700 to 1,000 μmol/L at the completion of the infusion is required for successful therapy. This was found to be correlated significantly with prognosis.[8,11,12] (In N.J.'s experience, superior results are more likely to be achieved with levels in excess of 1,500 μmol/L). Unfortunately, for reasons undetermined at the present time, there is inter- and intrapatient variability, and consistent high levels with each course are infrequent (hence the desirability to attain a more than adequate threshold level at 4 to 6 hours). This variability, inter alia, may account for the diverse and conflicting results in published studies.[13,14] When administered as a so-called block of therapy or an intensified course, an optimum schedule comprises four to eight doses at 10- to 14-day intervals; when administered in combination with other agents, generally doxorubicin, the interval between courses may be reduced to 3 to 4 weeks.

MTX should be administered with appropriate attention to the pretherapy requirements and diligent monitoring of the decay curve. Infection and pathologic accumulation of fluid (ascites and pleural and pericardial effusions) where MTX may sequestrate and result in a prolonged delay in excretion are contraindications. MTX levels in excess of an institute's specific designated values at specified times portend potential toxicity and should be treated immediately by increasing intravenous hydration and augmenting the dose of leucovorin. The decay curve has wide variability: 24 hours, 30 to 300 μmol/L; 48 hours, 3 to 30 μmol/L; and 72 hours, less than 0.3 μmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Algorithms are available for this purpose and should be consulted. Generally, these doses are administered as 10 mg of leucovorin intravenously or orally every 6 hours; however, levels in excess of 50 μmol/L at 24 hours, 10 μmol/L at 48 hours, and 0.5μmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose; for example, 100 mg/m^2 administered intravenously every 3 hours. Elevations or reductions in the doses continue to be implemented throughout the period of rescue in accordance with the algorithms until the MTX level is $\leq$ 0.1 μmol/L (some centers will discontinue leucovorin at $\leq$ 0.3 μmol/L).

One of the earliest signs of toxicity is oliguria followed by anuria, presumably resulting from MTX precipitation in the renal tubules. Reduced renal clearance is generally accompanied by gastrointestinal, hematologic, and hepatic dysfunction. Toxicity can become excessive, but with appropriate early recognition and management, the

DOI: 10.1200/JCO.2007.14.7793
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patient will usually recover. Additional measures to abort life-threatening toxicity comprise hemodialysis using a high-flux dialyzer and carboxypeptidase-G2. This cleaves MTX into inactive metabolites. Irreversible toxicity, however, is rare, and several centers now advocate treatment on an outpatient basis.[15] Patients should be cautioned to avoid over-the-counter medication that may interfere with MTX excretion (eg, ibuprofen, aspirin, and acetaminophen). Certain antibiotics, such as penicillin, may cause a similar problem.

MTX is cell cycle specific for the S phase. It acts by inhibiting the conversion of dihydrofolate to tetrahydrofolate and prevents the transfer of 1- carbon donors. This surceases the folate pool, thwarts the formation of purine and thymidine, and ultimately disrupts DNA replication. However, the dose of MTX in osteosarcoma is many-fold the conventional dose, and it probably operates via a different mechanism. One simplistic possibility resides in the transport mechanism. MTX and leucovorin share a common carrier, called the reduced folate carrier, for entry into cells. This transport mechanism is present in normal cells but not in resistant cancer cells. High-dose MTX enters normal cells via the carrier mechanism and, by virtue of its high extracellular concentration, also resistant malignant cells through diffusion over time (concentration $\times$ time). Leucovorin also enters normal cells via the carrier mechanism, but unlike MTX, is prohibited from entry into malignant cells because of its relatively extracellular low serum concentration and the absence of a carrier mechanism. It rescues normal cells while malignant cells, in the absence of intracellular leucovorin, self-destruct.

The ability of MTX-L to destroy malignant osteosarcoma cells led to intensive investigations to determine its metabolic and biochemical activities. Studies evaluated blood, urinary, and cerebral spinal fluid levels in children after high-dose MTX infusions, examined age-related different pharmacokinetics, and discovered contaminants and specific metabolites during therapy and degradation and clearance of the antifolate.[16,17] Age may be important in terms of effective dosage.[17] Nonetheless, the mechanism of action of MTX-L in osteosarcoma remains an enigma.

Attempts to enhance the efficacy of MTX-L in osteosarcoma by extending the period of administration from 4 or 6 hours to 24 hours or prolonging the interval before initiating the rescue program have not been successful.[18] Similarly, administering a high MTX dose by the intra-arterial route, which produces a higher local concentration as opposed to the intravenous route, does not enhance the efficacy of its therapeutic application (Jaffe et al,[19] also republished in this issue of the *Journal of Clinical Oncology*). Others have administered MTX in even higher doses, seemingly without increasing its efficacy. Thus the regimen seems to have reached the limits of its utility with the conventional intravenous high dose (10 to 12.5 g/m^2).

Despite the enigma of its mechanism of action, successes achieved with the regimen in osteosarcoma spawned attempts to develop new and similar agents to treat the disease. This led to the rational synthesis of folate analogs that differ from MTX in transport, metabolism, and intracellular targets. These include trimetrexate and pemetrexed. Trimetrexate with simultaneous leucovorin has shown some promising activity in the context of phase II trials of patients with recurrent disease, but it remains unknown whether it can add to the efficacy of upfront MTX-L. Similarly, pemetrexed administered with simultaneous folic acid supplementation is in the midst of phase II testing in osteosarcoma as part of Children's Oncology Group clinical trials.

With the republication of the study comparing intra-arterial and intravenous MTX-L after a quarter century[19] and 36 years after the first demonstration of its efficacy,[20] it is appropriate to recognize and finally affirm its contribution to the treatment of osteosarcoma.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Manuscript writing: Norman Jaffe, Richard Gorlick
Final approval of manuscript: Norman Jaffe, Richard Gorlick

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Cancer Investigation, 9(1) 53–60 (1991)

CLINICAL TRIALS
Ellin Berman, M.D., Editor

Methotrexate and Childhood Leukemia

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INTRODUCTION

Cancer takes second place only to accidents as the most common cause of death in children less than 15 years of age. Leukemia is the most common malignancy in this age group, the majority of childhood leukemia being acute lymphoblastic leukemia (ALL). It accounts for approximately 20–25% of pediatric malignant disease.

Forty years ago, the folic acid antagonist aminopterin was first used to treat ALL in children (1). These ensuing four decades have seen major advances in the treatment of ALL, such that patients with "standard risk" ALL now have a 60–70% chance of long-term disease-free survival. This progress has been made possible, in part, through multiple clinical and laboratory trials. More recently, large multi-institutional treatment protocols (e.g., POG, CCSG, BFM), aimed at better defining groups of patients at risk for relapse and improving treatment further, have been organized.

This article reviews the use of methotrexate (MTx) in ALL. It is not intended to be a complete overview of the clinical and experimental use of MTx. That is impossible in the space permitted. For example, a recent review of the clinical use of high-dose MTx (2) had 150 references alone, and there are entire texts devoted to folic acid antagonists as drugs (3). Rather, it is our purpose to note some highlights of ALL therapy using MTx and critically evaluate these studies in light of increasing knowledge on the metabolism of folates and folic acid antagonists. We also speculate on means of using MTx or its analogs in a better manner to enhance the efficacy of ALL therapy.

OVERVIEW OF METHOTREXATE DEVELOPMENT

Methotrexate (NSC 740) is one of several antibiotic or antineoplastic folic acid antagonists. It is one of the oldest and most frequently used antineoplastic agents. The initial development of antifolate compounds occurred in the late 1940s, only a few years after the isolation of folic acid from spinach in 1941 and its synthesis in 1945 (4–6). Although it was not until after 1950 that the role of folate compounds in one-carbon metabolism and DNA synthesis was appreciated, in the 1940s it was realized that folic acid could stimulate growth of cells and micro-organisms. Furthermore, the observation had been made that folic acid seemed to exacerbate the disease when administered to patients with ALL (the so-called acceleration phenomenon). Therefore, a series of folic acid analogs, called x-methyl folates, were synthesized for use as folate antagonists, but were found to have no antitumor activity.

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However, a new compound, aminopterin (4-aminopteroylglutamic acid), was found to be antagonist to folic acid regarding the growth of certain types of bacteria. This activity, coupled with the acceleration phenomenon, suggested to Dr. Sidney Farber, that administration of folic acid antagonists might be effective in inhibiting the growth of lymphoblasts. In 1948, Farber and his associates reported on the use of aminopterin in inducing temporary remissions in acute leukemia in children (1). They reported the results of clinical and hematological studies of 5 of 16 children treated with aminopterin. The children ranged in age from 2.5 to 7.5 years, and were treated with 0.5–1.0 mg of the drug i.m. daily. All had extensive disease which responded promptly to this treatment. Marked clinical improvement and decreased numbers of lymphoblasts were noted, but remissions were temporary. Liver extract, which is known to contain large amounts of reduced folates, was administered periodically in an attempt to reduce toxicity, mainly stomatitis (first use of *folate rescue*)! Additionally it was emphasized that the drug is toxic and may be productive of even greater disturbances (toxicity) than had been demonstrated in these studies.

In the 3-4 years following the introduction of aminopterin, other antimetabolites such as 6-mercaptopurine (6-MP) were synthesized (7). Mtx (amethopterin), which seemed to have more predictable toxicity than aminopterin, was used with more frequency than aminopterin and the concept of combination therapy was introduced. For example, in 1952 striking potentiating effects were found in leukemic mice given methotrexate and 8-azaguanine simultaneously (8). This combination effected 90-day survival in more than 10% of the 300 mice treated, as opposed to approximately 10-day survival time in untreated controls, 15-day survival in the 8-azaguanine group, and a 20–40-day survival in the Mtx-treated animals.

In the early 1950s, the combination of MTx administered with leucovorin (folinic acid) rescue was examined in studies by Goldin and co-workers (9). They presented evidence that giving leucovorin simultaneously with MTx allowed higher doses of MTx to be given, but a reduced antitumor effect was observed at equivalent toxicity. Administration of leucovorin subsequent to MTx increased the specificity of action of the MTx by reducing the toxicity to the host without altering the effectiveness of the drug against the tumor. In this circumstance too, the protection afforded by leucovorin to the host permitted using higher doses of MTx for host toxicity equivalent to lower doses of drug without rescue. The authors hypothesized that tumor tissue had less reserve to protect against the effects of MTx than the host. The increased survival time supported the theory that the tumor was less likely to be rescued with leucovorin.

In 1956, the question of the timing of the MTx dose in the treatment of the L1210 leukemia in mice was studied (10). Specifically, it was noted that the effectiveness of MTx was influenced by: (i) the time at which treatment was initiated following implantation of leukemic cells, (ii) spacing of the treatments, (iii) the number of treatments, (iv) the administration of leucovorin, and (v) the concentration of the leukemic inocolum. The conclusions reached by Goldin and his associates were that the optimal schedule of therapy and the optimal dose are not fixed, but were influenced by the parameters noted. The results emphasized the dual need in chemotherapy to (a) recognize the stage of the disease, and to (b) develop appropriate therapies for each stage. For example, animals with large amounts of disease responded better to smaller, more frequent dosage schedules rather than larger single doses of drug(s).

Modern therapy for ALL is divided into initial (induction) and continuation phases of therapy. Additionally, CNS prophylaxis is part of the modern ALL therapy. MTx is a mainstay in the latter two phases and in the early years, prior to vincristine and prednisone, also part of initial induction therapy. The next section reviews the use of MTx during these three components of therapy.

INDUCTION THERAPY

After initial observations by Farber and his colleagues (1) and the development of 6-MP (7), clinical trials evaluating MTx and 6-MP in children with ALL were done. Various treatment schedules were utilized in an effort to improve survival. Prior to the widespread use of vincristine (VCR) and prednisone for remission induction, the largest study to examine the efficacy of 6-MP and MTx in induction treatment of leukemia, was presented by the Acute Leukemia Group B (CALGB) in 1961 (11). There were three main phases to this study: First, Mtx (about 3 mg/m^2/day) was given for 4-10 weeks followed by 6-MP (3 mg/kg/day); second, 6-MP was followed by MTx, using the same doses of both drugs and similar schedule, and third, combination therapy was administered with both drugs given in full dosage. It was clear that the combination therapy was statistically better at inducing remissions (i.e., 59% vs. 47% for 6-MP and 29% for MTx). The combination chemotherapy also resulted in longer duration of remission. This study took 3 years to finish and accrued 318 patients. It involved 13

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institutions with patient entries varying from approximately 2.5 patients per month to 1 patient every 2 months among the various institutions. This study is therefore also noteworthy for documenting the effectiveness of multi-institutional participation in completing a protocol in a relatively short period of time.

Later in the 1960s, when it became clear that VCR and prednisone were effective in inducing remissions in approximately 90% of patients with ALL (12), the above mentioned study may have served as an impetus for the use of 6-MP and MTx in the continuation phase of ALL therapy. These drugs were the only other active agents that could be used without known long-term toxicity.

CONTINUATION THERAPY

It became clear relatively early that following successful remission induction and no further treatment that leukemia recurred in about six weeks. This led to the concept of a continuation phase of chemotherapy (also referred to as "maintenance"). MTx and 6-MP were the obvious candidates to be used for this phase of treatment because they were the only other known active agents. In 1965, the CALGB published a study of the efficacy of MTx during the continuation phase of therapy, after remission had been achieved with VCR and prednisone (12). Patients were randomized to receive either MTx 30 mg/m^2 i.m. twice weekly or MTx 3 mg/m^2 per day orally. The results of this study clearly indicated, that semiweekly MTx, given i.m. was superior to daily oral administration. No patient was in complete remission on daily oral MTx by approximately 500 days, but 20% of the patients treated with semiweekly MTx were in continuous complete remission at approximately 2 years. In a followup study (13), the biweekly MTx schedule was used, with oral or intramuscular MTx administration compared. There was no difference between the two methods, but the overall continuous complete remission rate was somewhat shorter than in the previous study.

In 1965, Frei and co-workers presented a study of remission continuation regimens involving 166 patients from 10 institutions (14). This study consisted of three arms: Daily 6-MP and MTx; daily 6-MP for a month alternating monthly with daily MTx, and; daily 6-MP and MTx with intrathecal MTx (see CNS Therapy below). This study showed that 6-MP and daily MTx was not superior to 6-MP alone and that daily MTx given long term was associated with severe toxic manifestations. This study and the 1965 CALGB report may have suggested the return to weekly MTx in combination with daily 6-MP for maintenance of remission in ALL, as did concomitant work done at the St. Jude Children's Research Hospital (15).

By the 1970s, MTx and 6-MP were clearly established as cornerstones of continuation therapy in ALL (reviewed in ref. 16). This time period also saw the development of protocols using intermediate dose MTx (IDMTx) (500–1000 mg/m^2) and/or high-dose MTx (HDMTx) ($\geq$ 1 g/m^2) with leucovorin rescue during the continuation phase of therapy. Increasing the dose of MTx was done in an effort to increase the cure rate and lower the morbidity associated with multimodality therapy, specifically eliminating cranial irradiation as part of CNS prophylaxis. Emerging data from these studies indicate that IDMTx with IT MTx may not be as effective as cranial irradiation in preventing CNS leukemia (17–19), but there appears to be lower incidence of testicular relapse in boys treated with IDMTx. These studies are also evidence that IDMTx or HDMTx regimens may prolong continuous complete marrow remission, at least in standard risk patients, and be more efficacious than other intensive treatment protocols in this respect. The rationale and use of IDMTx and HDMTx will be discussed below and compared with therapy with conventional doses of MTx. A current analysis of efficacy of IDMTx in therapy of ALL at a single institution has recently been published. It suggests that there may be a correlation between higher serum levels of MTx and improved outcome (20).

CNS DISEASE

With more effective treatment available for obtaining and then maintaining remission in ALL, new aspects of the disease, which previously were not recognized, became more prominent. The most troublesome of these was leukemic involvement of the central nervous system. In 1954, attention was drawn to neurologic abnormalities observed in 2 of 24 leukemic children treated with MTx, cortisone, and ACTH (21). These two children, in bone marrow remission, developed a syndrome characterized by obesity of the Froelich's type, and a variety of neurologic signs. These children were also found to have blast cells in the cerebrospinal fluid (CSF). Treatment for the disease was with 0.025 and 0.036 mg of IT MTx for 2 and 6 days, respectively, which resulted in decreased number of blasts in the CSF. Unfortunately, the clinical status of the patients did not improve.

In 1958, the results of pharmacokinetic studies of MTx given intracisternally to dogs and a clinical series on the treatment of the neurological manifestations of leukemia

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in children with intrathecal MTx were presented by Whiteside and co-workers from the Memorial Sloan Kettering Institute (22). In this landmark report, a dose of 10–15 mg/m^2 MTx given intrathecally was established as safe, with encouraging clinical results in the children so treated, despite their advanced CNS leukemia.

With the realization that systemic leukemia could be treated effectively with combination chemotherapy and that CNS leukemia could be treated with intrathecal chemotherapy and/or radiation to the cerebrospinal axis, the concept of "total therapy" for ALL emerged (23). The ultimate goal of therapy was to establish a significant cure rate for childhood acute lymphoblastic leukemia by using multimodality therapy including combination chemotherapy and specific CNS prophylaxis. In a series of studies from St. Jude's during the mid 1960s through the 1970s it became clear, that given 2400 rads to the craniospinal axis with or without IT MTx, combined with the use of systemic chemotherapy, would allow a significant proportion of children to remain in prolonged continuous complete remission, and 50% or more of these patients could be considered cured.

Based upon efficacy, the standard prophylaxis against CNS leukemia consisted of 2400 rads to the cranium plus ITMTx given early in remission. As noted above, several studies in the 1970s examined the use of IDMTx with leucovorin rescue to prevent CNS leukemia, but CNS disease was not as well prevented (17–19). In light of more recent pharmacology data, several questions remain, however, about the MTx dose schedule used in these studies (see next section). Similarly, it is not clear, when and how much leucovorin to administer. Since leucovorin is rapidly converted to 5-methyltetrahydrofolate, which does accumulate in the spinal fluid, it is conceivable that giving too much leucovorin systemically might significantly decrease the efficacy of even IDMTx for the treatment of CNS disease (i.e., selectively rescue the CNS space) because MTx enters poorly compared with the folate (24,25).

In efforts to decrease both acute and chronic neurotoxicity associated with ITMTx and radiation (see below), more recent studies have evaluated prophylactic CNS therapy using triple intrathecal therapy (TIT) with MTx, Ara-C, and hydrocortisone without radiation. In patients with standard risk ALL, treated with TIT, the incidence of overt CNS leukemia is approximately 5%, a value similar to that obtained with IT MTx and radiation (26). However, it should be noted that long-term followup with psychological testing has not yet proved the presumed safety of this regimen, although it seems to have less severe late effects than cranial irradiation. It is not known, whether TIT is superior to IT MTx alone as CNS prophylaxis.

MTx TOXICITY

With more effective therapy being available for ALL, the concern about toxicity from its treatment becomes increasingly important. MTx has been shown to have a variety of toxicities, including mucositis, marrow suppression, and hepatitis-like liver dysfunction (27). Other acute toxicities, including renal toxicity with HDMTx are also well described (28). A MTx pneumonitis has been described, but the pathogenesis is not clear (29). However, the greatest concern relates to its CNS effects. This has been reviewed in recent years by several investigators (30–32). It is known that patients with inborn errors of folate metabolism can have a variety of neurological and developmental abnormalities. Decreased folate content has been documented in the brain of rats and subhuman primates treated with MTx (33,34). It is therefore plausible that MTx, by inducing a folate-deficient state, could also cause toxicity to the CNS. The CNS effects of chronic MTx use have recently been reviewed (35).

PHARMACOLOGY

Methotrexate is a 4-amino-substituted pterin compound, and is the prototypic folic acid antagonist. It has been shown to have a reliable absorption in doses up to 30 mg/m^2, but at least one study (reviewed in ref. 36) has suggested that variation in the rate of drug absorption between individual patients may affect treatment outcome.

Methotrexate is a weak organic acid with low lipid solubility and therefore does not diffuse readily across membranes or into the CSF. It equilibrates slowly in the total body water, including third spaces. The blood–brain barrier limits the penetration of MTx into the CSF such that it achieves only 1–2% of the concomitant plasma concentration. MTx is protein-bound in blood to a variable degree. Other drugs that bind to plasma proteins (e.g., ASA) can therefore affect this protein binding and alter the free plasma concentration of MTx.

When given in low doses, MTx is cleared through the kidneys almost totally unchanged. Other weak organic acids compete with MTx for secretion in the kidney tubules, and this can delay excretion of MTx. Giving higher doses of MTx results in the formation of 7-OHMTx, primarily via hepatic aldehyde oxidase. This metabolite of MTx has a much longer half-life than MTx

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and is about five times less soluble in an acidic environment. It may be this metabolite that precipitates in the renal tubules, contributing to the renal damage associated with high-dose MTx regimens.

Methotrexate transport is thought to be via a facilitated diffusion or carrier-mediated process. The K_m for transport is generally 1–10 μM (37). However, most studies on folate transport have been done in folate replete cells grown in large amounts of folic acid (100–1000-fold plasma folate). More recently, a membrane receptor for folate and MTx has been characterized (38–40). Several cell types have been shown to have a high affinity membrane receptor (K_m about 1 nM) for folate and at least one cell type can accumulate folate such that the intracellular concentration is several hundredfold that of the medium after only 4 at 37 °C. The carrier has also been described kinetically (41), and tentatively identified with photoaffinity labelling (42). It has not yet been purified and studied in a cell-free system. As a folate analog, MTx has been found to compete for binding the same membrane receptor and transport system as reduced folate (37,40,41). The relationship between the carrier and receptor is not known.

Classically, the main site of action and the cause–effect relationship of MTx activity have been considered to be the inhibition of the enzyme dihydrofolate reductase (DHFR). MTx has at least a thousandfold increased affinity over folate for DHFR. This presumed mechanism of action has lead to several hypotheses to explain cellular resistance to MTx; most often gene amplification resulting in overproduction of DHFR. This was part of the rationale for the development of HDMTx therapy. No large studies, however, support the superiority of HDMTx over other more conventional doses (43–45) and no patients are reported with significant gene amplification for DHFR in their tumor compared with results of studies in vivo. Thus clinical resistance is not yet explained, but may be related to more recent observations concerning the interrelationship of folate and MTx transport and accumulation, as described above. The time of drug exposure is clearly a more important parameter than the concentration in the medium or plasma, once a certain critical concentration is reached. In IDMTx and HDMTx regimens, prolonged exposure to MTx at levels above this critical concentration is achieved by giving a single large dose, but it could also be reached by using conventional doses give over a period of time. In other words, once the plasma or extracellular concentration of the drug exceeds the K_m for the transport system, increasing the extracellular concentration further may add little to the observed cytotoxicity and may only contribute to acute toxicity in vivo. Since plasma folate is only $1–5 \times 10^{-8}$M and folate requirements for normal growth and development are only 2–3 μg/kg per day (e.g., a 1 M^2 child needs only 60–90 μg/day) then giving even 30 mg/m^2 (i.e., 30,000 μg) MTx is giving a large excess of MTx compared with folate. The availability of leucovorin and the often used attitude of "more is better" however, may lead to the continued use of HDMTx.

Intracellular folate is predominently in a polyglutamate form, with 5-methyltetrahydrofolylpolyglutamates predominating. These polyglutamates are not only storage forms for folate, but are actually the preferred substrates for many folate-requiring enzymes (see 46). They have been shown to be important for normal cellular growth. This seems especially true for two steps in de novo purine synthesis, which depend on folate cofactors, with folate polyglutamates shown to be the preferred substrates. It is only in 1973 that MTx was shown to be a substrate for folate polyglutamate synthetase in the rat liver (47) and 1977 in human liver (48). More recent studies using high-performance liquid chromatography (HPLC) methodology has confirmed that MTx is also a substrate for folate polyglutamate synthetase in humans (49) and that these metabolites of MTx are potent inhibitors of de novo purine synthesis (50). Oxidized folates, generated secondary to MTx inhibition of DHFR, may also inhibit de novo purine synthesis. MTx polyglutamates have a long intracellular half-life compared with MTx. They may also inhibit folate accumulation at the level of membrane transport or polyglutamation.

7-OHMTx, which is found primarily after HDMTx therapy, is not a potent inhibitor of DHFR. Its actions intracellularly are not fully understood. It has been shown, however, that 7-OHMTx exists in mono- or polyglutamated forms, and that it can interfere with both folate and MTx metabolism and transport. Since 7-OH MTx is less soluble than MTx and has a significantly longer half-time, it may be responsible for some toxicities associated with MTx therapy, as noted above.

In summary, after 40 years of relatively empiric use, it is now becoming clear that MTx interferes with folate homeostasis at many different sites. The more recent knowledge of MTx and its metabolites having multiple cellular targets, not only DHFR, has improved our understanding of biochemical events in the cell cycle in normal and neoplastic cells, and may be used to develop new strategies in the clinical use of MTx. These additional sites of action are also potential targets for the development of new folic acid antagonists having enzymes other

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than DHFR as prime target. With better understanding of the mechanism of resistance to MTx, strategies to block tumor resistance are also conceivable.

CONCLUSION AND FUTURE PROSPECTS

In the last four decades, tremendous advances have been made in the treatment of ALL in childhood. These advances were brought about first by the increased availability of new and effective drugs and second, by a better understanding of their mechanism of action. Recent advances have been made through better supportive care (e.g., broad spectrum antibiotics, transfusion support, parenteral nutrition and dialysis when necessary, etc.), which have allowed more intensive therapy to be tested. The latest innovations include the use of bone marrow transplantation, either allogeneic or autologous with purging of the marrow, based upon recognition of leukemic cell features using immunological methods. The definition of blast cell phenotypes indicative of relatively resistant disease defines prognosis and the need for more intensive or specific therapy.

Current therapy for ALL is still toxic and prolonged, and more importantly, still results in treatment failure in up to half of all patients. Even a third of patients with standard risk ALL will relapse. The continuation phase of therapy is the one that has received least attention in recent years, yet it is here that the majority of treatment failures occur. New strategies are clearly needed in order to reach the goal of curing the majority of children with ALL. The explosive increase in the knowledge of the biology of lymphoblasts offers hope that new therapies can be devised aimed at specific targets on the malignant lymphoblasts such as surface antigens, growth factors, or their receptors or other regulators of biochemical pathways in cell proliferation and/or differentiation. The possibility of suppressing oncogenes is also intriguing, and the role of the multiple drug resistance (MDR) gene and its product, the cell membrane p-glycoprotein, in drug resistance will also need to be further defined. New immunologic and molecular biology techniques might also be used to better detect minimal residual leukemia and thereby allow more reliable evaluation of response to treatment, especially during the continuation phase. However, from a pharmacological view, better scheduling of known effective agents such as MTx and 6-MP should also be done.

In the last 20 years, changes in the treatment of ALL have primarily evolved around therapeutic intensification through the use of many cytotoxic agents in order to improve outcome and reduce toxicities. Progress has been much slower during this time than the previous two decades. Using more intensive therapy with multiple agents at higher doses has not clearly improved results, and very few effective agents have been introduced. In this light, it is of note that there is still no concensus as to the optimal use of MTx in ALL. Goldin showed more than 30 years ago that the status of the disease was critical in determining schedule and dose (i.e., small frequent doses during ties of bulk disease). This is shown clinically when continuous infusion low-dose MTx (total dose less than 100 mg) caused remissions in children who previously failed more conventional MTx and multiagent therapy (51). This study of Djerassi and colleagues supports the laboratory model that time of exposure as well as total dose of drug is the most important variable. Thus, since a prime principle of cancer therapy has been in use effective drugs to a maximum tolerated dose (MTD), instead of intensifying chemotherapy using many agents (e.g., anthracyclines, alkylating agents) which will decrease the amount of other drugs that can be administered in any given time, it may be important to simply increase or improve the use of the known effective drugs. This is especially true for MTx and 6-MP, because much empiric use has shown their relative safety and laboratory capabilities now allow alternative, novel scheduling to be developed and tested clinically. An example of such a protocol is a POG pilot study in which parenteral 6-MP and MTx are given intensively every other week for 12 cycles (52). At the end of this 6-month period, children would have received 20 times the MTx and $>$ 2 times 6-MP compared with standard regimens and even 4 times more MTx than earlier protocols using IDMTx. Also, the MTx and 6-MP have been timed to optimize the synergy of the two agents found in vivo (53,54). Additionally, the 6-MP, given parenterally, may be more effective than just two times the dose and may offer additional CNS protection. Modifications such as found in this protocol allow intensification of ALL therapy through increased administration of known effective agents as a rationale basis for developing pharmacological strategies in forthcoming years.

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RIGHTSLINK

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Fusilev safely and effectively. See full prescribing information for Fusilev.

FUSILEV (levoleucovorin calcium) powder, injection, lyophilized, for solution for intravenous use

Initial U.S. Approval: 1952 (d,l-leucovorin), 2008 (levoleucovorin)

INDICATIONS AND USAGE

Fusilev is a folate analog. (1)

Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma. (1)

Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists. (1)

Limitations of Use

Fusilev is not approved for pernicious anemia and megaloblastic anemias. Improper use may cause a hematologic remission while neurologic manifestations continue to progress. (1.1)

DOSAGE AND ADMINISTRATION

Fusilev Rescue After High-Dose Methotrexate Therapy

Do not administer intrathecally. (2.1)

Fusilev is dosed at one-half the usual dose of the racemic form. (2.1)

Fusilev rescue recommendations are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours. Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion. Determine serum creatinine and methotrexate levels at least once daily. Continue Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) until the methotrexate level is below 5 x 10^{-8} M (0.05 micromolar). The Fusilev dose may need to be adjusted. (2.3)

DOSAGE FORMS AND STRENGTHS

Each 50 mg single-use vial of Fusilev contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol. (16) It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride for Injection, USP. (2.5, 11)

CONTRAINDICATIONS

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid. (4)

WARNINGS AND PRECAUTIONS

Due to Ca^{++} content, no more than 16 mL (160 mg) of levoleucovorin solution should be injected intravenously per minute. (5.1)

Fusilev enhances the toxicity of fluorouracil. (5.2,7)

Concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for Pneumocystis carinii pneumonia in HIV patients was associated with increased rates of treatment failure in a placebo-controlled study. (5.3)

ADVERSE REACTIONS

Allergic reactions were reported in patients receiving Fusilev. (6.2)

Vomiting (38%), stomatitis (38%) and nausea (19%) were reported in patients receiving Fusilev as rescue after high dose methotrexate therapy. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Spectrum Pharmaceuticals, Inc. at 1-877-387-4538 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

To report SUSPECTED ADVERSE REACTIONS, contact at or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

DRUG INTERACTIONS

Fusilev may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible patients. (7)

Revised: 09/2008

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*** Sections or subsections omitted from the full prescribing information are not listed**

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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FULL PRESCRIBING INFORMATION

1. INDICATIONS AND USAGE

- Fusilev™ is a folate analog.
- Fusilev rescue is indicated after high-dose methotrexate therapy in osteosarcoma.
- Fusilev is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists.

1.1 Limitations of Use

-
- Fusilev is not approved for pernicious anemia and megaloblastic anemias secondary to the lack of vitamin B_{12}. Improper use may cause a hematologic remission while neurologic manifestations continue to progress.

2. DOSAGE AND ADMINISTRATION

2.1 Administration Guidelines

Fusilev is dosed at **one-half** the usual dose of the racemic form.
Fusilev is indicated for intravenous administration only**. Do not administer intrathecally.**

2.2 Co-administration of Fusilev with other agents

Due to the risk of precipitation, do not co-administer Fusilev with other agents in the same admixture.

2.3 Fusilev Rescue After High-Dose Methotrexate Therapy

The recommendations for Fusilev rescue are based on a methotrexate dose of 12 grams/m^2 administered by intravenous infusion over 4 hours (see methotrexate package insert for full prescribing information). Fusilev rescue at a dose of 7.5 mg (approximately 5 mg/m^2) every 6 hours for 10 doses starts 24 hours after the beginning of the methotrexate infusion.
Serum creatinine and methotrexate levels should be determined at least once daily. Fusilev administration, hydration, and urinary alkalinization (pH of 7.0 or greater) should be continued until the methotrexate level is below 5 x 10^{-8} M (0.05 micromolar). The Fusilev dose should be adjusted or rescue extended based on the following guidelines.
Table 1 Guidelines for Fusilev Dosage and Administration

Clinical Situation	Laboratory Findings	Fusilev Dosage and Duration
Normal Methotrexate Elimination	Serum methotrexate level approximately 10 micromolar at 24 hours after administration, 1 micromolar at 48 hours, and less than 0.2 micromolar at 72 hours	7.5 mg IV q 6 hours for 60 hours (10 doses starting at 24 hours after start of methotrexate infusion).
Delayed Late Methotrexate Elimination	Serum methotrexate level remaining above 0.2 micromolar at 72 hours, and more than 0.05 micromolar at 96 hours after administration.	Continue 7.5 mg IV q 6 hours, until methotrexate level is less than 0.05 micromolar.
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	Serum methotrexate level of 50 micromolar or more at 24 hours, or 5 micromolar or more at 48 hours after administration, OR; a 100% or greater increase in serum creatinine level at 24 hours after methotrexate administration (e.g., an increase from 0.5 mg/dL to a level of 1 mg/dL or more).	75 mg IV q 3 hours until methotrexate level is less than 1 micromolar; then 7.5 mg IV q 3 hours until methotrexate level is less than 0.05 micromolar.

Patients who experience delayed early methotrexate elimination are likely to develop reversible renal failure. In addition to appropriate Fusilev therapy, these patients require continuing hydration and urinary alkalinization, and close monitoring of fluid and electrolyte status, until the serum methotrexate level has fallen to below 0.05 micromolar and the renal failure has resolved.
Some patients will have abnormalities in methotrexate elimination or renal function following methotrexate administration, which are significant but less severe than the abnormalities described in the table above. These abnormalities may or may not be associated with significant clinical toxicity. If significant clinical toxicity is observed, Fusilev rescue should be extended for an additional 24 hours (total of 14 doses over 84 hours) in subsequent courses of therapy. The possibility that the patient is taking other medications which interact with methotrexate (e.g., medications which may interfere with methotrexate elimination or binding to serum albumin) should always be reconsidered when laboratory abnormalities or clinical toxicities are observed.
Delayed methotrexate excretion may be caused by accumulation in a third space fluid collection (i.e., ascites, pleural effusion), renal insufficiency, or inadequate hydration. Under such circumstances, higher doses of Fusilev or prolonged administration may be indicated.

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Although Fusilev may ameliorate the hematologic toxicity associated with high dose methotrexate, Fusilev has no effect on other established toxicities of methotrexate such as the nephrotoxicity resulting from drug and/or metabolite precipitation in the kidney.

2.4 Dosing Recommendations for Inadvertent Methotrexate Overdosage

Fusilev rescue should begin as soon as possible after an inadvertent overdosage and within 24 hours of methotrexate administration when there is delayed excretion. As the time interval between antifolate administration [e.g., methotrexate] and Fusilev rescue increases, Fusilev's effectiveness in counteracting toxicity may decrease. Fusilev 7.5 mg (approximately 5 mg/m^2) should be administered IV every 6 hours until the serum methotrexate level is less than 10^{-8} M.

Serum creatinine and methotrexate levels should be determined at 24 hour intervals. If the 24 hour serum creatinine has increased 50% over baseline or if the 24 hour methotrexate level is greater than 5×10^{-6} M or the 48 hour level is greater than 9×10^{-7} M, the dose of Fusilev should be increased to 50 mg/m^2 IV every 3 hours until the methotrexate level is less than 10^{-8} M. Hydration (3 L/day) and urinary alkalinization with $NaHCO_3$ should be employed concomitantly. The bicarbonate dose should be adjusted to maintain the urine pH at 7.0 or greater.

2.5 Reconstitution and Infusion Instructions

- Prior to intravenous injection, the 50 mg vial of Fusilev for Injection is reconstituted with 5.3 mL of 0.9% Sodium Chloride Injection, USP to yield a levoleucovorin concentration of 10 mg per mL. Reconstitution with Sodium Chloride solutions with preservatives (e.g. benzyl alcohol) has not been studied. The use of solutions other than 0.9% Sodium Chloride Injection, USP is not recommended.

- The reconstituted 10 mg per mL levoleucovorin contains no preservative. Observe strict aseptic technique during reconstitution of the drug product.

- Saline reconstituted levoleucovorin solutions may be further diluted, immediately, to concentrations of 0.5 mg/mL to 5 mg/mL in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Initial reconstitution or further dilution using 0.9% Sodium Chloride Injection, USP may be held at room temperature for not more than a total of 12 hours. Dilutions in 5% Dextrose Injection, USP may be held at room temperature for not more than 4 hours.

- Visually inspect the reconstituted solution for particulate matter and discoloration, prior to administration. CAUTION: Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if cloudiness or precipitate is observed.

- No more than 16 mL of reconstituted solutions (160 mg of levoleucovorin) should be injected intravenously per minute, because of the calcium content of the levoleucovorin solution.

3. DOSAGE FORMS AND STRENGTHS

Fusilev is supplied in sterile, single-use vials containing 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.

4. CONTRAINDICATIONS

Fusilev is contraindicated for patients who have had previous allergic reactions attributed to folic acid or folinic acid.

5. WARNINGS AND PRECAUTIONS

5.1 Rate of Administration

Because of the Ca^{++} content of the levoleucovorin solution, no more than 16 mL (160 mg of levoleucovorin) should be injected intravenously per minute.

5.2 Potential for Enhanced Toxicity with 5-Fluorouracil

Fusilev enhances the toxicity of 5-fluorouracil. Deaths from severe enterocolitis, diarrhea, and dehydration have been reported in elderly patients receiving weekly *d,l*-leucovorin and 5-fluorouracil.

5.3 Potential for interaction with trimethoprim-sulfamethoxazole

The concomitant use of *d,l*-leucovorin with trimethoprim-sulfamethoxazole for the acute treatment of Pneumocystis carinii pneumonia in patients with HIV infection was associated with increased rates of treatment failure and morbidity in a placebo-controlled study.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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6. ADVERSE REACTIONS

6.1 Clinical Studies Experience

Since clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The following table presents the frequency of adverse reactions which occurred during the administration of 58 courses of high dose methotrexate 12 grams/m^2 followed by Fusilev rescue for osteosarcoma in 16 patients age 6-21. Most patients received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate.

Table 2 Adverse Reactions

Body System/Adverse Reactions	Number (%) of Patients with Adverse Reactions		Number (%) of Courses with Adverse Reactions	
	(N =16)		(N = 58)	
	All	Grade 3+	All	Grade 3+
Gastrointestinal				
Stomatitis	6 (37.5)	1 (6.3)	10 (17.2)	1 (1.7)
Vomiting	6 (37.5)	0	14 (24.1)	0
Nausea	3 (18.8)	0	3 (5.2)	0
Diarrhea	1 (6.3)	0	1 (1.7)	0
Dyspepsia	1 (6.3)	0	1 (1.7)	0
Typhlitis	1 (6.3)	1 (6.3)	1 (1.7)	1 (1.7)
Respiratory				
Dyspnea	1 (6.3)	0	1 (1.7)	0
Skin and Appendages				
Dermatitis	1 (6.3)	0	1 (1.7)	0
Other				
Confusion	1 (6.3)	0	1 (1.7)	0
Neuropathy	1 (6.3)	0	1 (1.7)	0
Renal function abnormal	1 (6.3)	0	3 (5.2)	0
Taste perversion	1 (6.3)	0	1 (1.7)	0
Total number of patients	9 (56.3)		2 (12.5)	
Total number of courses	25 (43.1)		2 (3.4)	

The incidence of adverse reactions may be underestimated because not all patients were fully evaluable for toxicity for all cycles in the clinical trials. Leukopenia and thrombocytopenia were observed, but could not be attributed to high dose methotrexate with Fusilev rescue because patients were receiving other myelosuppressive chemotherapy.

6.2 Postmarketing Experience

Since adverse reactions from spontaneous reports are provided voluntarily from a population of uncertain size, it is not always possible to estimate reliably their frequency or establish a causal relationship to drug exposure. Spontaneously reported adverse reactions collected by the WHO Collaborating Center for International Drug Monitoring in Uppsala Sweden have yielded seven cases where levoleucovorin was administered with a regimen of methotrexate. The events were dyspnea, pruritus, rash, temperature change and rigors. For 217 adverse reactions (108 reports) where levoleucovorin was a suspected or interacting medication, there were 40 occurrences of “possible allergic reaction.”

7. DRUG INTERACTIONS

Folic acid in large amounts may counteract the antiepileptic effect of phenobarbital, phenytoin and primidone, and increase the frequency of seizures in susceptible children. It is not known whether folinic acid has the same effects. However, both folic and folinic

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acids share some common metabolic pathways. Caution should be taken when taking folinic acid in combination with anticonvulsant drugs.
Preliminary human studies have shown that small quantities of systemically administered leucovorin enter the CSF, primarily as its major metabolite, 5-methyltetrahydrofolate (5-MTHFA). In humans, the CSF levels of 5-MTHFA remain 1-3 orders of magnitude lower than the usual methotrexate concentrations following intrathecal administration.
Fusilev increases the toxicity of 5-fluorouracil *[see Warnings and Precautions (5.2)]*.

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. It is not known whether Fusilev can cause fetal harm when administered to a pregnant woman or if it can affect reproduction capacity. Animal reproduction studies have not been conducted with Fusilev. Fusilev should be given to a pregnant woman only if clearly needed.

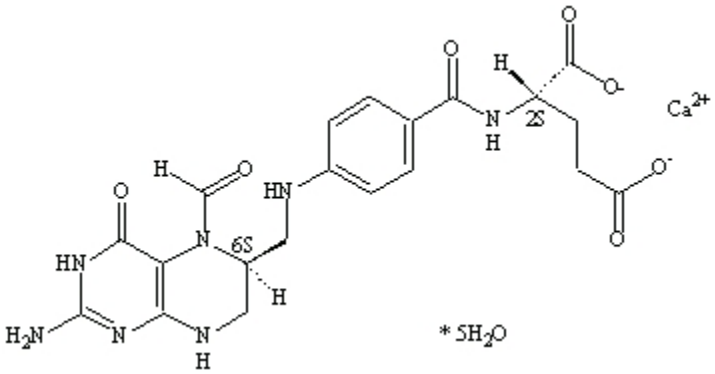
8.3 Nursing Mothers
It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Fusilev is administered to a nursing mother.

8.4 Pediatric Use
[See Clinical Studies (14)]

8.5 Geriatric Use
Clinical studies of Fusilev in the treatment of osteosarcoma did not include subjects aged 65 and over to determine whether they respond differently from younger subjects.

10. OVERDOSAGE
No data are available for overdosage with levoleucovorin.

11. DESCRIPTION
Levoleucovorin is the levo isomeric form of racemic *d,l*-leucovorin, present as the calcium salt. Levoleucovorin is the pharmacologically active isomer of leucovorin [(6-S)-leucovorin].
Fusilev for injection contains levoleucovorin calcium, which is one of several active, chemically reduced derivatives of folic acid. It is useful as antidote to the inhibition of dihydrofolate reductase by methotrexate. This compound has the chemical designation calcium (6S)-N-{4-[[(2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridinyl)methyl] amino]benzoyl}-L-glutamate pentahydrate. The molecular weight is 601.6 and the structural formula is:



Its molecular formula is: $C_{20}H_{21}CaN_7O_7 \cdot 5\ H_2O$.
Fusilev for injection is supplied as a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol per 50 mg vial.
Sodium hydroxide and/or hydrocholoric acid are used to adjust the pH during manufacture. It is intended for intravenous administration after reconstitution with 5.3 mL of sterile 0.9% Sodium Chloride Injection, USP [*See Dosage and Administration (2.5)*]

12. CLINICAL PHARMACOLOGY

12.1 Mechanism Of Action
Levoleucovorin is the pharmacologically active isomer of 5-formyl tetrahydrofolic acid. Levoleucovorin does not require reduction by the enzyme dihydrofolate reductase in order to participate in reactions utilizing folates as a source of “one-carbon” moieties. Administration of levoleucovorin can counteract the therapeutic and toxic effects of folic acid antagonists such as methotrexate, which act by inhibiting dihydrofolate reductase.

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12.2 Pharmacodynamics

Levoleucovorin is actively and passively transported across cell membranes. In vivo, levoleucovorin is converted to 5-methyltetrahydrofolic acid (5-methyl-THF), the primary circulating form of active reduced folate. Levoleucovorin and 5-methyl-THF are polyglutamated intracellularly by the enzyme folylpolyglutamate synthetase. Folylpolyglutamates are active and participate in biochemical pathways that require reduced folate.

12.3 Pharmacokinetics

The pharmacokinetics of levoleucovorin after intravenous administration of a 15 mg dose was studied in healthy male volunteers. After rapid intravenous administration, serum total tetrahydrofolate (total-THF) concentrations reached a mean peak of 1722 ng/mL. Serum (6S)-5-methyl-5,6,7,8-tetrahydrofolate concentrations reached a mean peak of 275 ng/mL and the mean time to peak was 0.9 hours. The mean terminal half-life for total-THF and (6S)-5-methyl-5,6,7,8-tetrahydrofolate was 5.1 and 6.8 hours, respectively.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment Of Fertility

No studies have been conducted to evaluate the potential of levoleucovorin for carcinogenesis, mutagenesis and impairment of fertility.

13.2 Animal Toxicology And/Or Pharmacology

The acute intravenous LD_{50} values in adult mice and rats were 575 mg/kg (1725 mg/m^2) and 378 mg/kg (2268 mg/m^2), respectively. Signs of sedation, tremors, reduced motor activity, prostration, labored breathing, and/or convulsion were observed in these studies. Anticipated human dose for each administration is approximately 5 mg/m^2, which represents a 3-log safety margin.

14. CLINICAL STUDIES

The safety and efficacy of Fusilev rescue following high-dose methotrexate were evaluated in 16 patients age 6-21 who received 58 courses of therapy for osteogenic sarcoma. High-dose methotrexate was one component of several different combination chemotherapy regimens evaluated across several trials. Methotrexate 12 g/m^2 IV over 4 hours was administered to 13 patients, who received Fusilev 7.5 mg every 6 hours for 60 hours or longer beginning 24 hours after completion of methotrexate. Three patients received methotrexate 12.5 g/m^2 IV over 6 hours, followed by Fusilev 7.5 mg every 3 hours for 18 doses beginning 12 hours after completion of methotrexate. The mean number of Fusilev doses per course was 18.2 and the mean total dose per course was 350 mg. The efficacy of Fusilev rescue following high-dose methotrexate was based on the adverse reaction profile. *[See Adverse Reactions (6)]*

16. HOW SUPPLIED/STORAGE AND HANDLING

Each 50 mg single-use vial of Fusilev for Injection contains a sterile lyophilized powder consisting of 64 mg levoleucovorin calcium pentahydrate (equivalent to 50 mg levoleucovorin) and 50 mg mannitol.
50 mg vial of freeze-dried powder – NDC 68152-101-00.
Store at 25° C (77 °F) in carton until contents are used. Excursions permitted from 15-30° C (59-86 °F). [See USP Controlled Room Temperature]. Protect from light.

SPECTRUM PHARMACEUTICALS

Manufactured for Spectrum Pharmaceuticals, Inc.
Irvine, CA 92618
Manufactured by Chesapeake Biological Laboratories, Inc.
Baltimore, MD 21230

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Form Approved OMB No. 0910-511. See Instructions for OMB Statement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
MEDICAL DEVICE USER FEE COVER SHEET

PAYMENT IDENTIFICATION NUMBER: (b)(4) [redacted]
Write the Payment Identification number on your check.

A completed cover sheet must accompany each original application or supplement subject to fees. If payment is sent by U.S. mail or courier, please include a copy of this completed form with payment. Payment and mailing instructions can be found at: http://www.fda.gov/oc/mdufma/coversheet.html

1. COMPANY NAME AND ADDRESS (include name, street address, city state, country, and post office code)

ARK DIAGNOSTICS INC
1190 BORDEAUX DRIVE
SUNNYVALE CA 94089
US

1.1 EMPLOYER IDENTIFICATION NUMBER (EIN)
*****3512

2. CONTACT NAME
KENNETH KASPER

2.1 E-MAIL ADDRESS
ken@ark-tdm.com

2.2 TELEPHONE NUMBER (include Area code)
408-747-0708

2.3 FACSIMILE (FAX) NUMBER (Include Area code)

3. TYPE OF PREMARKET APPLICATION (Select one of the following in each column; if you are unsure, please refer to the application descriptions at the following web site: http://www.fda.gov/oc/mdufma

Select an application type:
[X] Premarket notification(510(k)); except for third party
[ ] 513(g) Request for Information
[ ] Biologics License Application (BLA)
[ ] Premarket Approval Application (PMA)
[ ] Modular PMA
[ ] Product Development Protocol (PDP)
[ ] Premarket Report (PMR)
[ ] Annual Fee for Periodic Reporting (APR)
[ ] 30-Day Notice

3.1 Select a center
[X] CDRH
[ ] CBER

3.2 Select one of the types below
[X] Original Application

Supplement Types:
[ ] Efficacy (BLA)
[ ] Panel Track (PMA, PMR, PDP)
[ ] Real-Time (PMA, PMR, PDP)
[ ] 180-day (PMA, PMR, PDP)

4. ARE YOU A SMALL BUSINESS? (See the instructions for more information on determining this status)
[X] YES, I meet the small business criteria and have submitted the required qualifying documents to FDA
NO, I am not a small business

4.1 If Yes, please enter your Small Business Decision Number: SBD118420

5. FDA WILL NOT ACCEPT YOUR SUBMISSION IF YOUR COMPANY HAS NOT PAID AN ESTABLISHMENT REGISTRATION FEE THAT IS DUE TO FDA. HAS YOUR COMPANY PAID ALL ESTABLISHMENT REGISTRATION FEES THAT ARE DUE TO FDA?
[X] YES (All of our establishments have registered and paid the fee, or this is our first device, and we will register and pay the fee within 30 days of FDA's approval/clearance of this device.)
[ ] NO (If "NO," FDA will not accept your submission until you have paid all fees due to FDA. This submission will not be processed; see http://www.fda.gov/cdrh/mdufma for additional information)

6. IS THIS PREMARKET APPLICATION COVERED BY ANY OF THE FOLLOWING USER FEE EXCEPTIONS? IF SO, CHECK THE APPLICABLE EXCEPTION.
[ ] This application is the first PMA submitted by a qualified small business, including any affiliates
[ ] This biologics application is submitted under section 351 of the Public Health Service Act for a product licensed for further manufacturing use only
[ ] The sole purpose of the application is to support conditions of use for a pediatric population
[ ] The application is submitted by a state or federal government entity for a device that is not to be distributed commercially

7. IS THIS A SUPPLEMENT TO A PREMARKET APPLICATION FOR WHICH FEES WERE WAIVED DUE TO SOLE USE IN A PEDIATRIC POPULATION THAT NOW PROPOSES CONDITION OF USE FOR ANY ADULT POPULATION? (If so, the application is subject to the fee that applies for an original premarket approval application (PMA).
[ ] YES
[X] NO

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8. USER FEE PAYMENT AMOUNT SUBMITTED FOR THIS PREMARKET APPLICATION

(b)(4) 02-Jun-2011

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Journal of Clinical Pharmacy and Therapeutics

Journal of Clinical Pharmacy and Therapeutics (2011) 36, 237 245 doi:10.1111/j.1365 2710.2010.01179.x

PHARMACOKINETICS

Methotrexate pharmacokinetics in childhood acute lymphoblastic leukaemia: a prognostic value ?

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**Medical Pharmacology and Toxicology Department, Lapeyronie Hospital, University Hospital of Montpellier, †Department of Pediatric Oncology, Arnaud de Villeneuve Hospital, University Hospital of Montpellier, ‡Department of Statistics and Epidemiology, University Institute for Clinical Research, Biostatistic Laboratory, University Hospital of Montpellier, and §Clinical Pharmacokinetic Laboratory, Faculty of Pharmacy, University of Montpellier 1, Montpellier, France*

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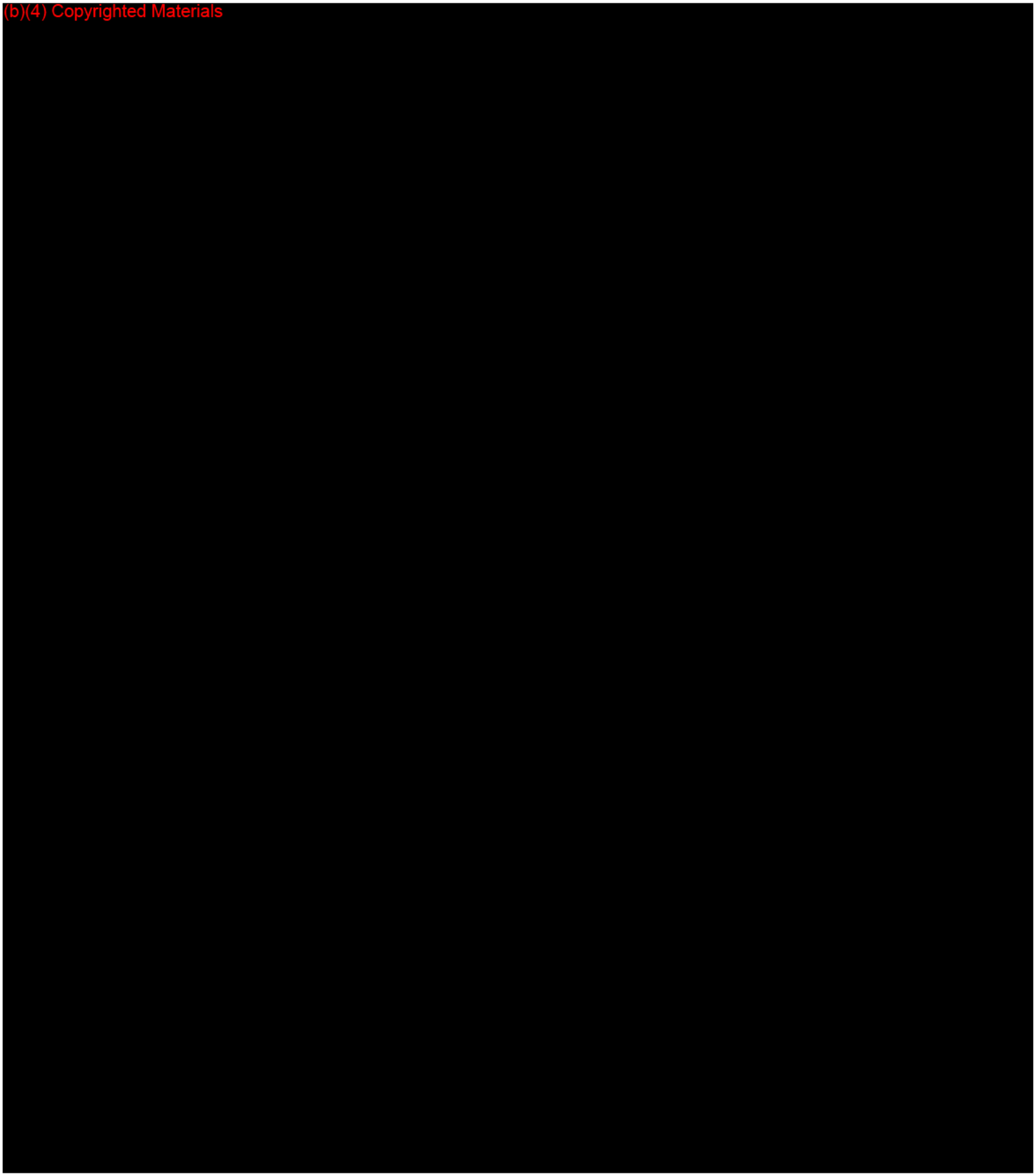

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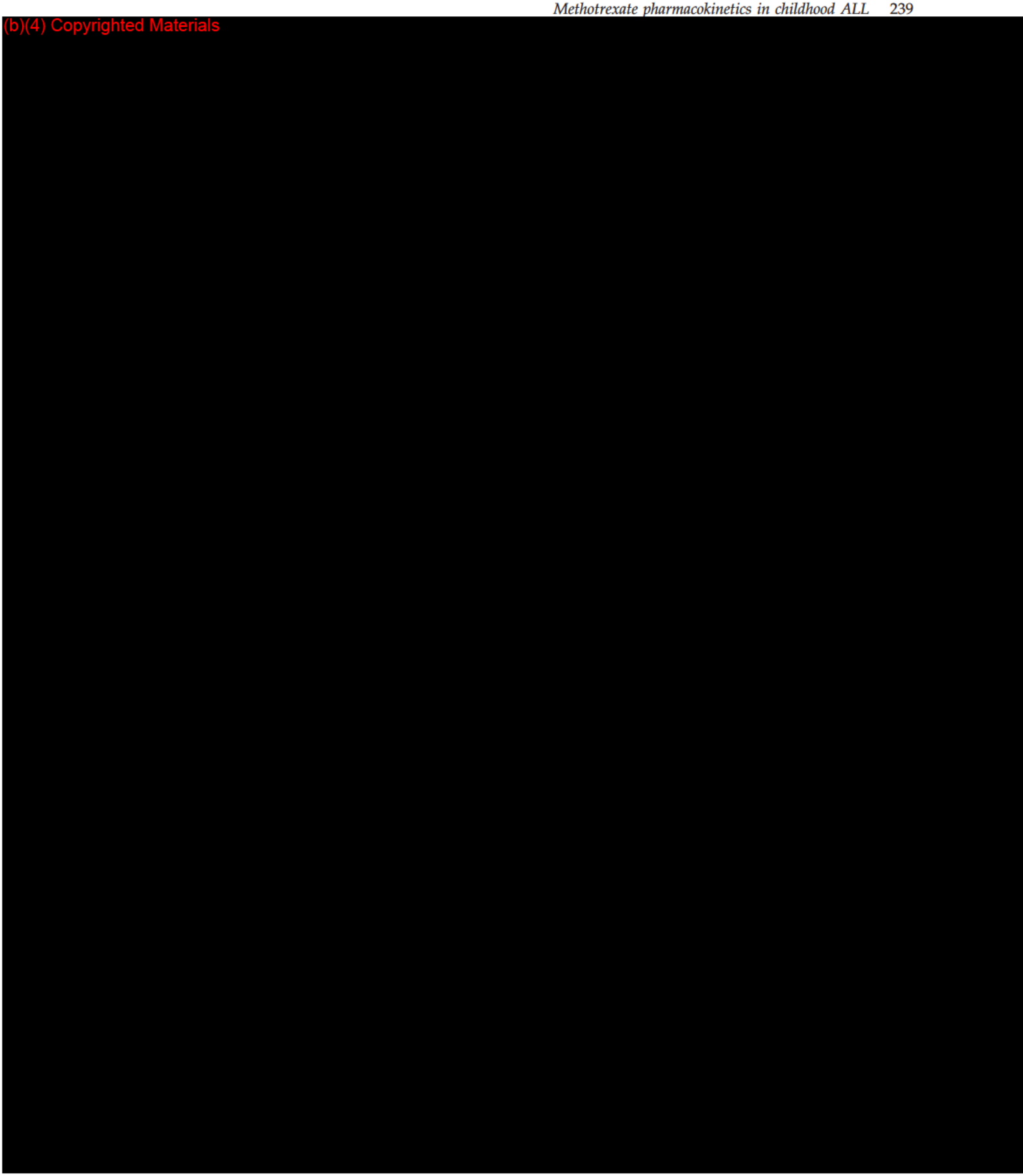

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Drug-induced osteoporosis

DANIEL J. MAZANEC, MD AND JOSEPH M. GRISANTI, MD

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Cancer Chemother Pharmacol (1983) 11: 29–32

Cancer Chemotherapy and Pharmacology

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Plasma Levels of 7-Hydroxymethotrexate after High-Dose Methotrexate Treatment

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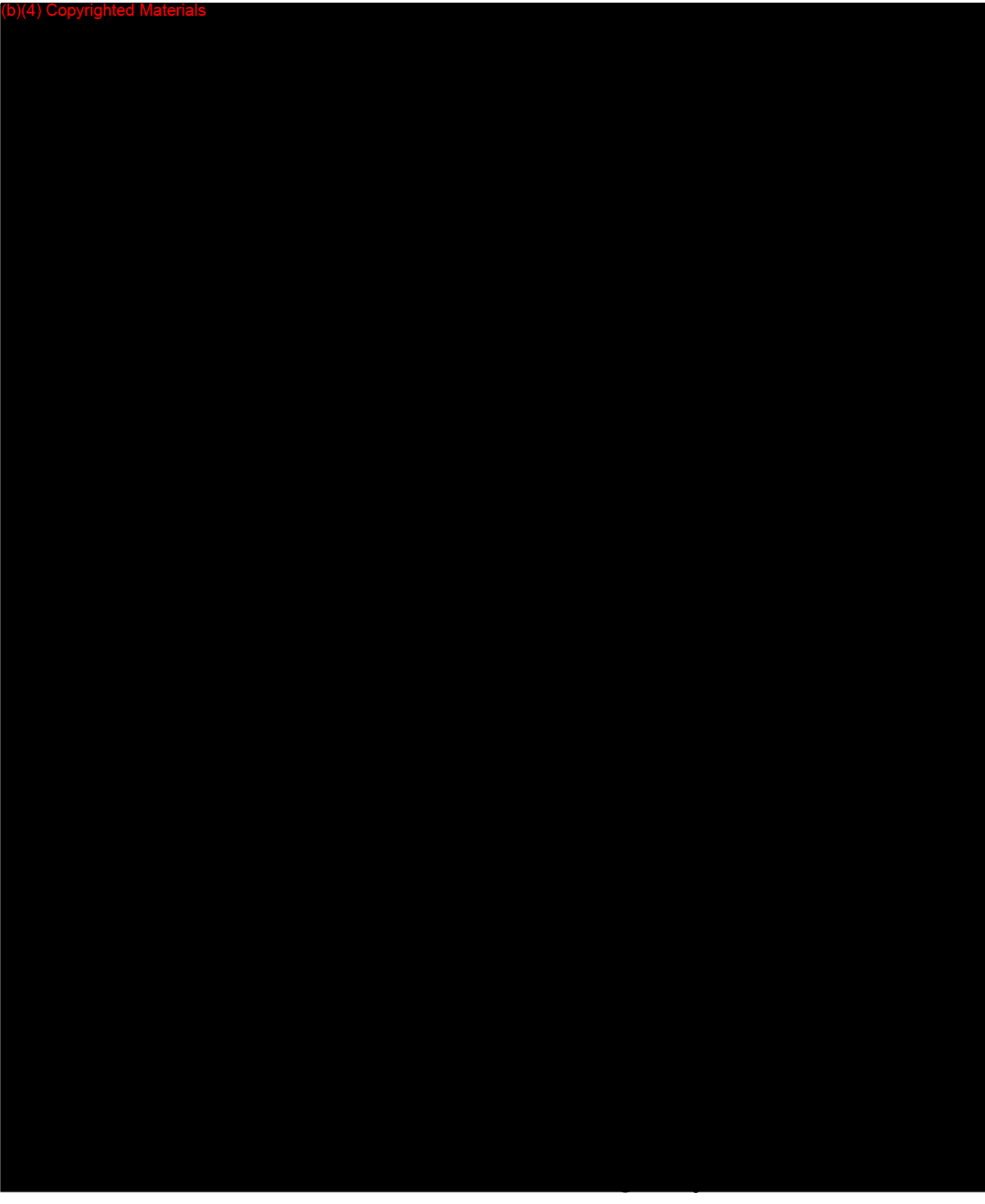
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Acknowledgments: The authors wish to thank F. Fein and N. Rameau for their assistance in translating and preparing the manuscript.

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ARK™ *Methotrexate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

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Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
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CE EC REP
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Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

© 2011, ARK Diagnostics, Inc. **Assay Kit REF 5026-0001-00**

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Name

ARK™ *Methotrexate Assay*

1 Intended Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

2 Summary and Explanation of the Test

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino]benzoyl]-L-glutamic acid], formerly Amethopterin, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1-3] Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Guidelines for methotrexate therapy with leucovorin rescue should be consulted.[1] Intermediate to high doses of methotrexate (approximately 35 mg/m^2 - 12 g/m^2) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[4-8]

3 Principles of the Procedure

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

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4 Reagents

REF	Product Description	Quantity/Volume
5026-0001-00	**ARK Methotrexate Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative, and stabilizers	**1 X 16 mL**
	Reagent R2 – Enzyme methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative, and stabilizers	**1 X 8 mL**

Reagent Handling and Storage

ARK Methotrexate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

5 Warnings and Precautions

- For In Vitro Diagnostic Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 Specimen Collection and Preparation for Analysis

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice.
- The sampling time of methotrexate will be dependent on dose, duration of infusion, and clinical status of the patient. Consult specific treatment protocols for sampling time.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
    - Sodium heparin
    - Lithium heparin
    - Potassium EDTA
- **DO NOT USE GEL SEPARATORS.** Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

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- Clarified specimens may be stored up to two weeks at 2 to 8°C. If testing will be delayed, specimens may be stored frozen (≤ -10°C) prior to being tested. Care should be taken to limit the number of freeze-thaw cycles. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C.
- **Handle all patient specimens as if they were potentially infectious.**

8 Procedure

Materials Provided
ARK Methotrexate Assay – REF 5026-0001-00

Materials Required – Provided Separately
ARK Methotrexate Calibrator – REF 5026-0002-00
Quality Controls – ARK Methotrexate Control – REF 5026-0003-00
ARK Methotrexate Dilution Buffer – REF 5026-0004-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Calibration
Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E, and F; test calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

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Quality Control (QC)
Laboratories should establish QC procedures for the ARK Methotrexate Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol
The range of the ARK Methotrexate calibration curve is 0.00 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the range of the calibration curve.

Manually dilute the high specimen or control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

	Sample Volume	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor.

9 Results

To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

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10 Limitations of Procedure

This assay is designed for use with serum or plasma only; refer to the sections Specimen Collection and Preparation for Analysis. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variability. See the section Expected Values below.

As with all analyte determinations, the methotrexate value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

IMPORTANT: Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay. These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid (DAMPA)[10-12] that result from metabolism of methotrexate by glucarpidase. DAMPA crossreacts with the methotrexate antibody used in this assay, and may continue to circulate for at least five to seven days before accurate measurements of serum methotrexate may return.[13] Oncologists on the clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA that would confuse the efforts of glucarpidase therapy.[13] While glucarpidase is well tolerated and rapidly reduces circulating MTX, delayed renal elimination of MTX can still be a problem for adult and elderly patients.[14]

11 Expected Values

Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors.[1,3] While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example),[15] concentrations may exceed 1000 µmol/L[16] with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.[17] For treatment of osteosarcoma,[16] the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, less than 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin in accordance with algorithms until the MTX level is <0.1 µmol/L. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.[1, 9] Some centers follow ≤ 0.10 µmol/L.[16, 18]

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From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.[1, 9, 19]

Clinical Situation	Laboratory Findings	
	Methotrexate Level (µmol/L)	Hours after administration
Normal Methotrexate Elimination	~10 ~1 <0.2	24 48 72
Delayed Late Methotrexate Elimination	>0.2 >0.05	72 96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50 ≥5 OR ≥100% increase in serum creatinine	24 48 24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs, [14, 19] for example vancomycin.[20] Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.[21-28]

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite,[15, 27] its molar ratio to methotrexate of up to approximately 100-fold,[29] and relative insolubility versus the parent drug,[14, 19] possible nephrotoxicity due to precipitation of the metabolite in renal tubules[29] may delay elimination of methotrexate itself.

Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.[14] Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.[13]

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12 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche/Hitachi 917 System.

Limit of Quantitation (LoQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report “not detected; concentration < LoD”
LoB < Result < LoQ	report “analyte detected; concentration < LoQ”
Result ≥ LoQ	report the result as measured

Measurement Range

The range of the ARK Methotrexate Assay calibration curve is 0.00 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen into the range of the calibration curve. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the calibration range.

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Recovery

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

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Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarized Immunoassay method (monoclonal FPIA)[30].

Methotrexate concentrations by FPIA ranged 0.02 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.00 to 1500 µmol/L. Results of the Passing-Bablok[31] regression analysis for the study are shown below (with 95% confidence limits) for 111 specimens within the calibration range as well as for all 157 specimens in the extended range.

Parameter	**Range 0.02 to 0.97 µM**		**Range 0.02 to 1440 µM**	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.01 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.971	(0.958 to 0.980)	0.998	(0.997 to 0.998)
Number of Samples	111	na	157	na

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.1 µmol/L, SD ≤0.01 at ≤0.1 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		**Methotrexate (~ 0.50 µmol/L)**	
Interfering Substance	**Interferent Concentration**	**Serum Control**	**Test**	**Serum Control**	**Test (% Control)**
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity

Methotrexate's metabolites, folate analogs and other compounds having structural similarity were tested to determine whether these compounds affect the quantitation of methotrexate concentrations using the ARK Methotrexate Assay. High levels of these compounds were spiked into serum pools containing low no methotrexate, 0.05 µmol/L or 0.50 µmol/L of methotrexate. The samples were analyzed and the methotrexate concentrations of samples containing interferent were compared to a serum control.

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Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 12 to 48 hours after HDMTX administration.[15, 27, 29, 32, 34-35]

Crossreactivity by 7-hydroxymethotrexate in the measurement of methotrexate was was determined for the ARK Methotrexate Assay by testing paired samples containing (1) both 0.05 µmol/L methotrexate and 5 µmol/L 7-hydroxymethotrexate and (2) both 0.50 µmol/L methotrexate and 50 µmol/L 7-hydroxymethotrexate in human serum.

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.

Crossreactivity to 2,4-Diamino-N[10]-methylpteroic acid (DAMPA)

As a minor metabolite of methotrexate, DAMPA is not expected to circulate at concentrations that would interfere in measurement of methotrexate.[33] However, following glucarpidase rescue therapy, the serum concentration of DAMPA can be substantial.[13, 14] The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for MTX cancer treatement due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

		Methotrexate Absent		Methotrexate Present 0.05 µmol/L		Methotrexate Present 0.50 µmol/L	
Compound	**Tested (µmol/L)**	**MTX (µmol/L)**	**Cross Reactivity (%)**	**MTX (µmol/L)**	**Cross Reactivity (%)**	**MTX (µmol/L)**	**Cross Reactivity (%)**
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

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Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

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Printed in USA
Revised June 2011
1600-0213-00 Rev 01draft

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ARK Diagnostics, Inc.

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ARK™ *Methotrexate Calibrator*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Calibrator must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	Calibrator	Calibrator

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1 Name

ARK™ ***Methotrexate Calibrator***

2 Intended Use

ARK Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Calibrator is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description		Quantity/Volume
5026-0002-00	**ARK Methotrexate Calibrators*** Methotrexate, buffer, bovine serum albumin, and preservatives		Dropper vials
	A	0.00 µmol/L	1 X 2 mL
	B	0.05 µmol/L	1 X 2 mL
	C	0.15 µmol/L	1 X 2 mL
	D	0.25 µmol/L	1 X 2 mL
	E	0.50 µmol/L	1 X 2 mL
	F	1.20 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0227, 0.0682, 0.1136, 0.2272, and 0.5453 µg/mL for Calibrators B to F respectively.

4 Standardization

There is no internationally recognized standard for Methotrexate. ARK Methotrexate Calibrators are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate.

5 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use**.**
- Do not mix calibrators from different lot numbers.
- Use each lot as a set.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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6 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Calibrators are ready to use. Mix each level by gentle inversion before dispensing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

7 Procedure

Calibration
Perform a full calibration (6- point) procedure; test calibrators in duplicate. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

8 Limitations of Procedure

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

9 Trademarks

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Printed in USA
Revised June 2011
1600-0214-00 Rev 01draft

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ARK Diagnostics, Inc.

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ARK™ *Methotrexate Control*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Control must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

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1 Name

ARK™ ***Methotrexate Control***

2 Intended Use

ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Control is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description	Quantity/Volume
5026-0003-00 Complete Set	**ARK Methotrexate Control*** Methotrexate, buffer, bovine serum albumin, and preservatives (nominal level)	Dropper vials
5026-0003-01 Calibration Range Controls[1]	**LOW** (0.07 µmol/L)	1 X 2 mL
	MID (0.40 µmol/L)	1 X 2 mL
	HIGH (0.80 µmol/L)	1 X 2 mL
5026-0003-02 High Range Controls[2]	**5** µmol/L	1 X 2 mL
	50 µmol/L	1 X 2 mL
	500 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0318, 0.1818, 0.3636, 2.272, 22.72, and 227.2 µg/mL respectively.

Each laboratory should establish its own ranges for each new lot of controls.

[1] Calibration range controls may be obtained separately as a set, REF 5026-0003-01.

[2] High range controls may be obtained separately as a set, REF 5026-0003-02.

4 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use**.**
- Do not mix controls from different lot numbers.
- Use each lot as a set.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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5 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Controls are ready to use. Mix each level by gentle inversion before dispensing.
- High range controls must be diluted prior to testing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

6 Limitations of Procedure

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

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Revised June 2011
1600-0215-00 Rev 01draft

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ARK Diagnostics, Inc.

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ARK™ *Methotrexate Dilution Buffer*

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

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1 Name

ARK™ ***Methotrexate Dilution Buffer***

2 Intended Use

ARK Methotrexate Dilution Buffer is intended for dilution of specimens containing high concentrations of methotrexate for the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Dilution Buffer is comprised of a synthetic protein matrix. Its composition is equivalent to Calibrator A (zero), ARK Methotrexate Calibrator, REF 5026-0002-00.

REF	Product Description	Quantity/Volume
5026-0004-00	**ARK Methotrexate Dilution Buffer** Buffer, bovine serum albumin, and preservatives	**1 X 25 mL**

4 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use**.**

5 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Dilution Buffer is ready to use. Mix by gentle inversion before dispensing.
- Store at 2-8°C. Use prior to the expiration date. Once opened, store at 2-8°C for 12 months within the expiration date.

6 Procedure – Manual Dilution Protocol

The calibration range of the ARK Methotrexate calibration curve is 0.00 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the range of the calibration curve.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Manually dilute the high specimen or high range control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

Sample Volume		Dilution Matrix Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor. To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

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ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised June 2011
1600-0216-00 Rev 01draft

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Br. J. Cancer (1991), 64, 405-410 © Macmillan Press Ltd., 1991

A randomised trial comparing combination chemotherapy using mitomycin C, mitozantrone and methotrexate (3M) with vincristine, anthracycline and cyclophosphamide (VAC) in advanced breast cancer

T.J. Powles, A.L. Jones, I.R. Judson, J.R. Hardy & S.E. Ashley

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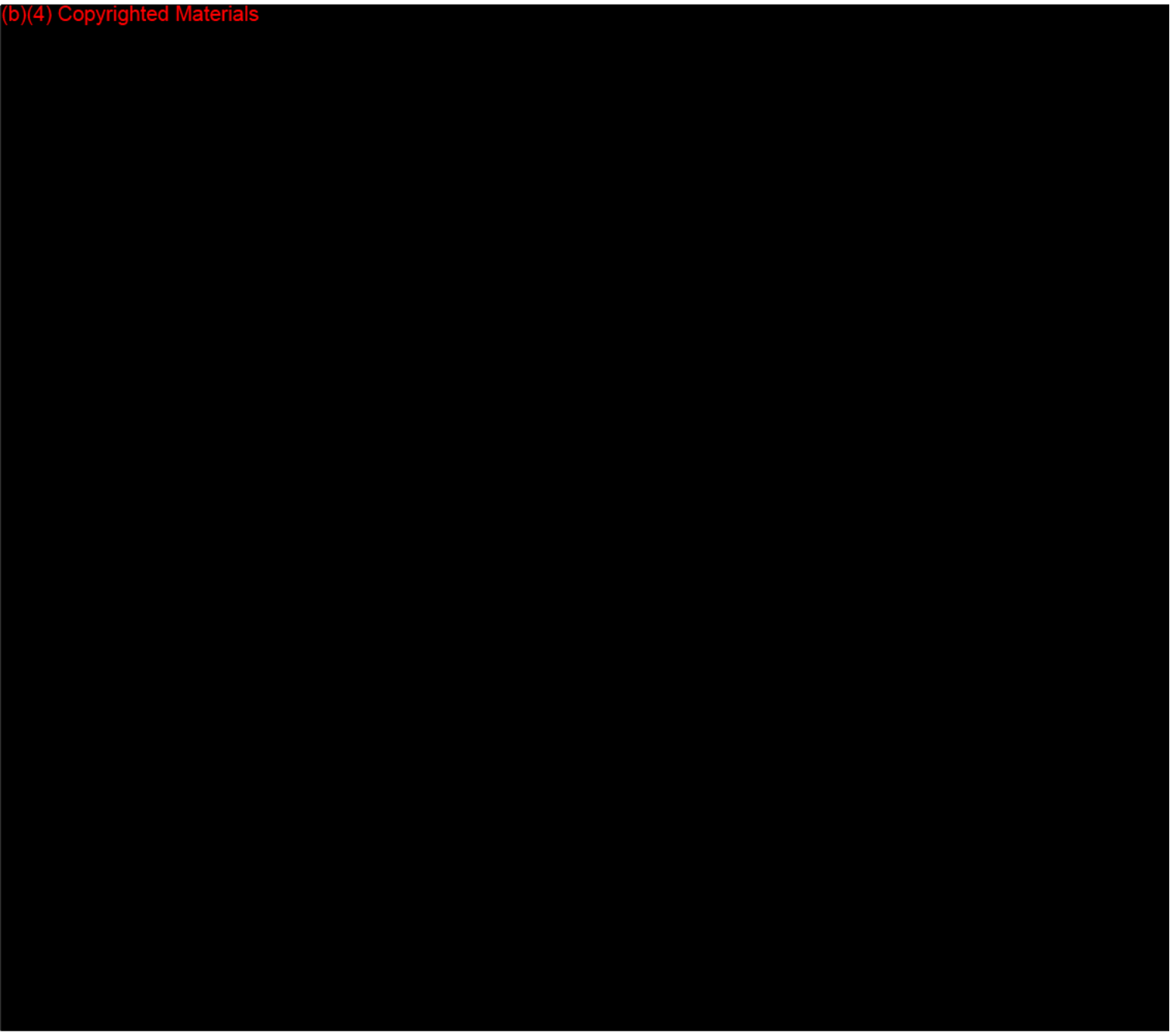

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MATTERS ARISING

Maternal age and the risk of developing ankylosing spondylitis

We read with interest the article by Weinreich and colleagues.[1] They showed that transgenic mice born to mothers aged 8 months or older had a significantly lower frequency of murine ankylosing enthesopathy than mice born to younger mothers. They speculated that an age related increase in maternal antibody levels resulted in increasing protection of offspring against a ubiquitous, potentially arthritogenic, micro-organism. In humans, as in the mouse model, environmental factors must influence the development of ankylosing spondylitis (AS). We sought evidence that the age of conception in women with AS influenced the risk of their offspring developing disease.

We collected data from 3473 patients with AS, regarding the age, and AS status, of relatives. Nine hundred and sixty two were female, male: female ratio 2.6:1. Six hundred and sixty five were recruited from the Royal National Hospital for Rheumatic Diseases (RNHRD), 2779 from the National Ankylosing Spondylitis Society (NASS), and 29 were relatives of RNHRD patients or NASS members, but were not RNHRD patients or NASS members themselves. Those from the RNHRD have been confirmed, by a rheumatologist, as having AS according to the New York criteria.[2] The diagnosis of AS in the overall patient population has been validated in three separate cohorts in which 100% of 146 patients had AS (personal communications M Brown, Oxford), 92.7% of 330 patients had AS (personal communications K Gomez, Bath), and 96% of 50 patients had AS.[3] Data were available for 39 pairs in which both mother and offspring had AS (21 mother:daughter pairs, 18 mother:son pairs). There were no families with data available for more than one mother:child pair. The higher than expected ratio of mother:daughter to mother:son pairs is the subject of an ongoing investigation. From our data base we chose 39 control pairs, where the mother had AS and the offspring did not, by matching the offspring for sex and age to the nearest year. We were able to match the offspring for birth rank in 37 cases (20 mother:daughter pairs, 17 mother:son pairs). The offspring were not matched for sibship size. The maternal ages at birth of offspring are shown (see table 1). Using paired *t* tests, we compared maternal ages at childbirth in the groups where the offspring had AS, and where the offspring did not have AS. The difference in the maternal age at childbirth was significant only when comparing mothers of sons with AS and mothers of sons without AS ($p = 0.04$; difference in mean maternal age at childbirth = 2.78 years). Mothers with AS, whose sons had AS, had given birth at a older age than those whose sons did not have AS.

Unlike the situation in the murine model, we find no evidence that increasing maternal age in humans is protective against the development of AS. However, the number of pairs available for our analysis was small. The influence of age at conception, on the development of AS in the offspring, remains an interesting area that deserves further attention.

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Table 1 Age of mothers with AS at birth of children, with AS and without AS

	AS mother: AS child mean (SD) maternal age at child birth	*AS mother: non AS child mean (SD) maternal age at child birth*
All mothers (n=39)	26.79 (4.14)	25.56 (4.98)
Mothers of daughters (n=21)	25.67 (3.37)	27.62 (5.17)
Mothers of sons (n=18)	28.11 (4.64)	25.33 (4.59)

LETTER TO THE EDITOR

Methotrexate and triamterene—a potentially fatal combination?

A 57 year old woman had been treated for several years with diclofenac 150 mg/day, atenolol 50 mg/day, and triamterene/hydrochlorthiazide 50/25 mg/day for rheumatoid arthritis (RA) and hypertension. Her doctor started treatment with methotrexate 5 mg/week for active RA. At the start of methotrexate treatment her full blood count was normal except for a haemoglobin of 10.1 g/dl. Renal and liver function were not checked. She weighed 68.5 kg. After one month her haemoglobin had fallen to 8.0 g/dl and her white cell count to 3.6×10^9/l and she developed mouth ulcers, dyspnoea, and easy bruising. She continued taking methotrexate, but after a month of these symptoms was admitted to hospital as an emergency.

On examination she was pale, tachypnoeic, and dehydrated with extensive mucosal ulceration. Investigations showed a pancytopaenia with renal impairment (Hb 4.1 g/dl, mean cell volume 89.5 fl, white cell count 3.1×10^9/l, neutrophils 2.0×10^9/l, lymphocytes 0.5×10^9/l, platelets 31×10^9/l, serum urea 20.8 mmol/l, and creatinine 148 μmol/l). Urine analysis, liver function tests, and chest *x* ray were unremarkable. Plasma folate was low at 1.6 μg/l (reference range 1.9—9.0). Cultures of body fluids were subsequently sterile.

She was treated for presumed methotrexate induced bone marrow suppression, with blood products and antimicrobials, discontinuation of usual medication, and, on haematological advice, oral folic acid 10 mg/day. Her white cell count fell over the next 48 hours (2.2×10^9/l, neutrophils 1.3×10^9/l), and a bone marrow biopsy showed features consistent with partially treated methotrexate induced megaloblastosis. Oral folinic acid 15 mg four times daily was substituted for folic acid and five days later her full blood count and renal function had returned to normal (Hb 10.5 g/dl, white cell count 6.1×10^9/l, platelets 268×10^9/l, serum urea 2.8 mmol/l, and creatinine 90 μmol/l).

Methotrexate is a folate antagonist, competing with folate for active transport into cells and competitively inhibiting dihydrofolate reductase (DHFR). The risk of marrow toxicity may be increased by co-prescribing other folate antagonists.[1]

Triamterene is a potassium sparing diuretic, structurally similar to folate, with anti-folate activity. It inhibits DHFR in vivo [2] and in vitro[3] and inhibits folic acid absorption in the rat.[4] Patients treated with high doses of triamterene for ascites may develop megaloblastic anaemia, which responds to folinic acid treatment.[3 5] Thus, there are good theoretical reasons for this patient developing marrow toxicity as a result of an interaction between methotrexate and triamterene. Long term triamterene treatment may have rendered her folate deficient, the two drugs may have had a synergistic effect on DHFR, or both of these mechanisms may have been involved.

The lack of information on pre-treatment renal function means that the possibility that toxicity developed as a result of reduced renal clearance of methotrexate cannot be excluded. Hypertension or the combination of medication may have resulted in compromised renal function. However, the fact that the serum urea was increased out of proportion to the creatinine, and both parameters returned to normal with rehydration suggests that her abnormal renal function may have been secondary to dehydration consequent upon her illness, rather than the cause of it.

In addition to this suspected drug interaction, this case highlights several other important points about the prescribing of methotrexate. It should be given under specialist supervision with baseline and regular monitoring of full blood count, renal and liver function.[6] The patient and doctor should be warned about possible side effects and advised to stop the drug should suspected side effects develop. Extra caution is required in patients with renal impairment[7] or receiving nephrotoxic drugs.[1] Finally folic acid has no place in the management of methotrexate induced pancytopaenia, the reduced form of folate, folinic acid, should be given as soon as the condition is suspected.[8]

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Treatment of Osteosarcoma of the Extremities With the T-10 Protocol, With Emphasis on the Effects of Preoperative Chemotherapy With Single-Agent High-Dose Methotrexate: A Scandinavian Sarcoma Group Study

By Gunnar Sæter, Thor A. Alvegård, Inkeri Elomaa, Anna E. Stenwig, Teddy Holmström, and Øyvin P. Solheim

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Journal of Clinical Oncology, Vol 9, No 10 (October), 1991: pp 1766-1775

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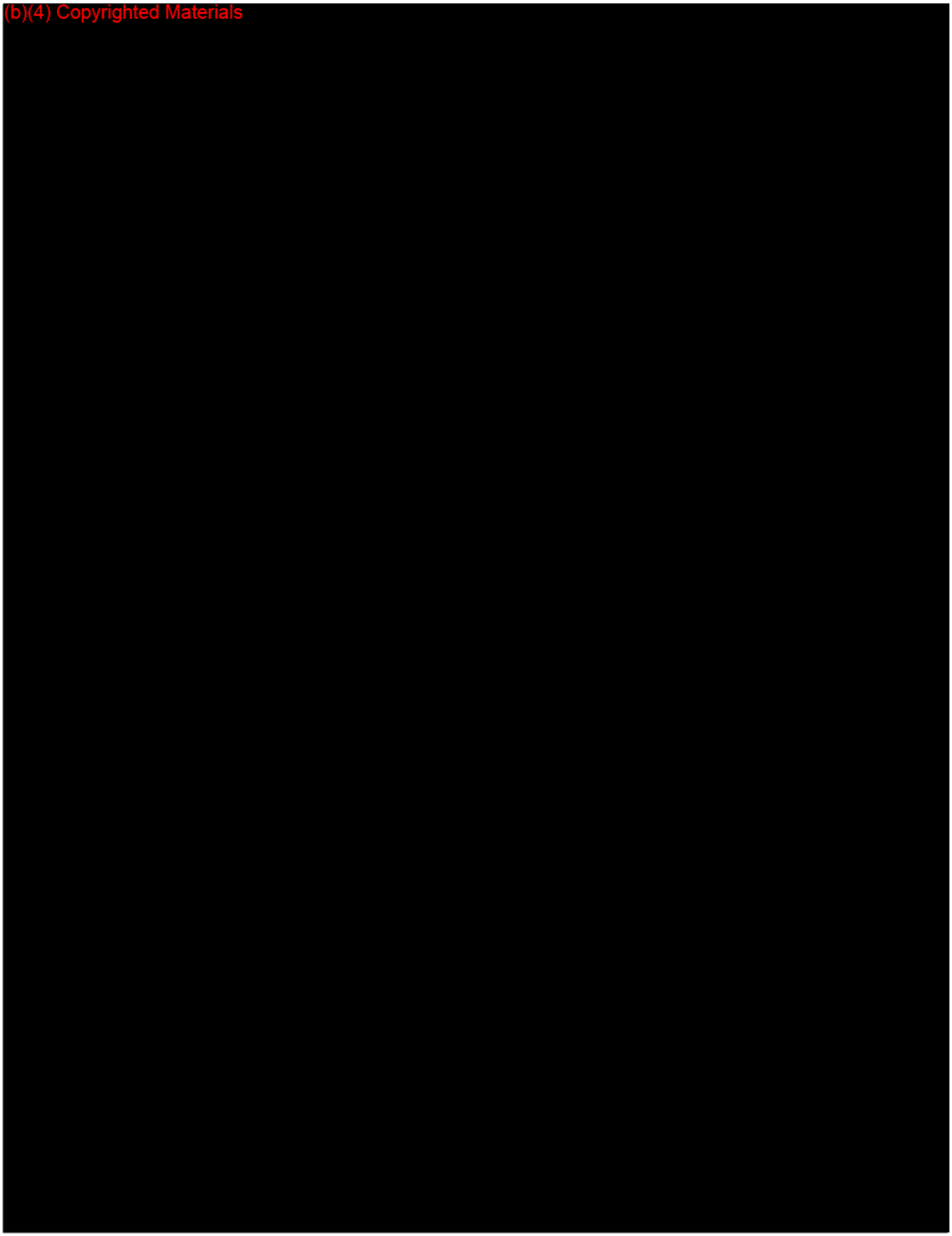

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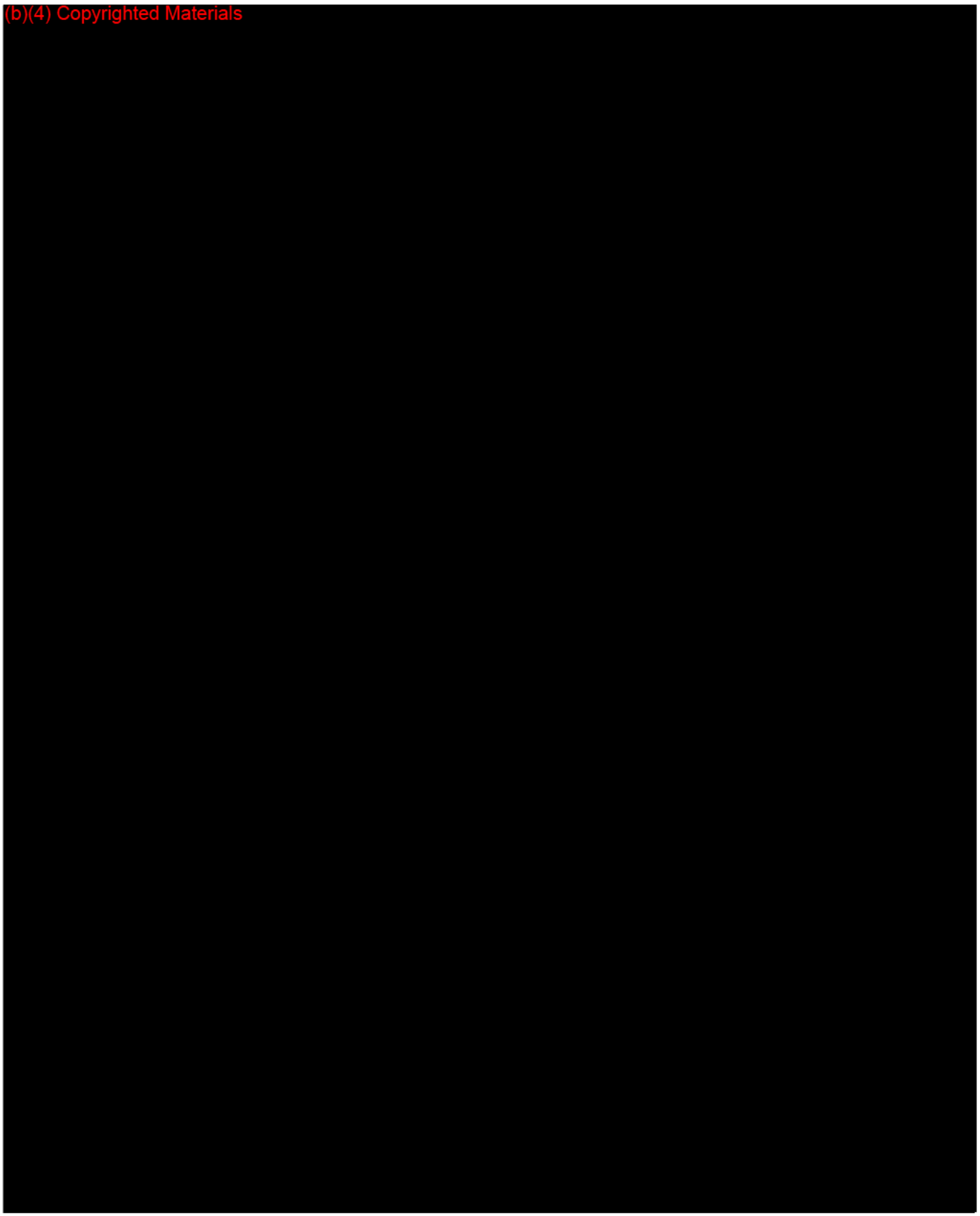

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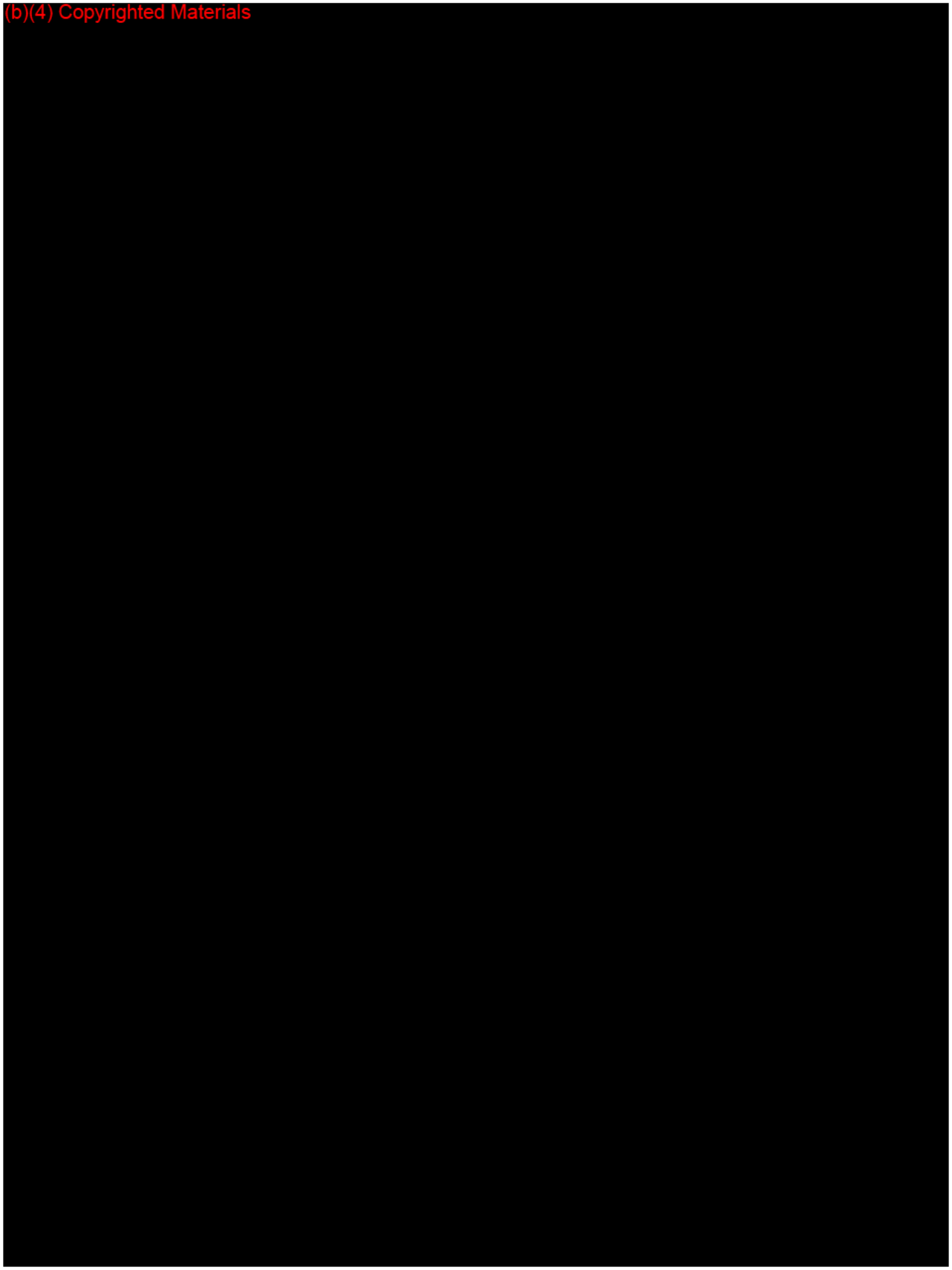

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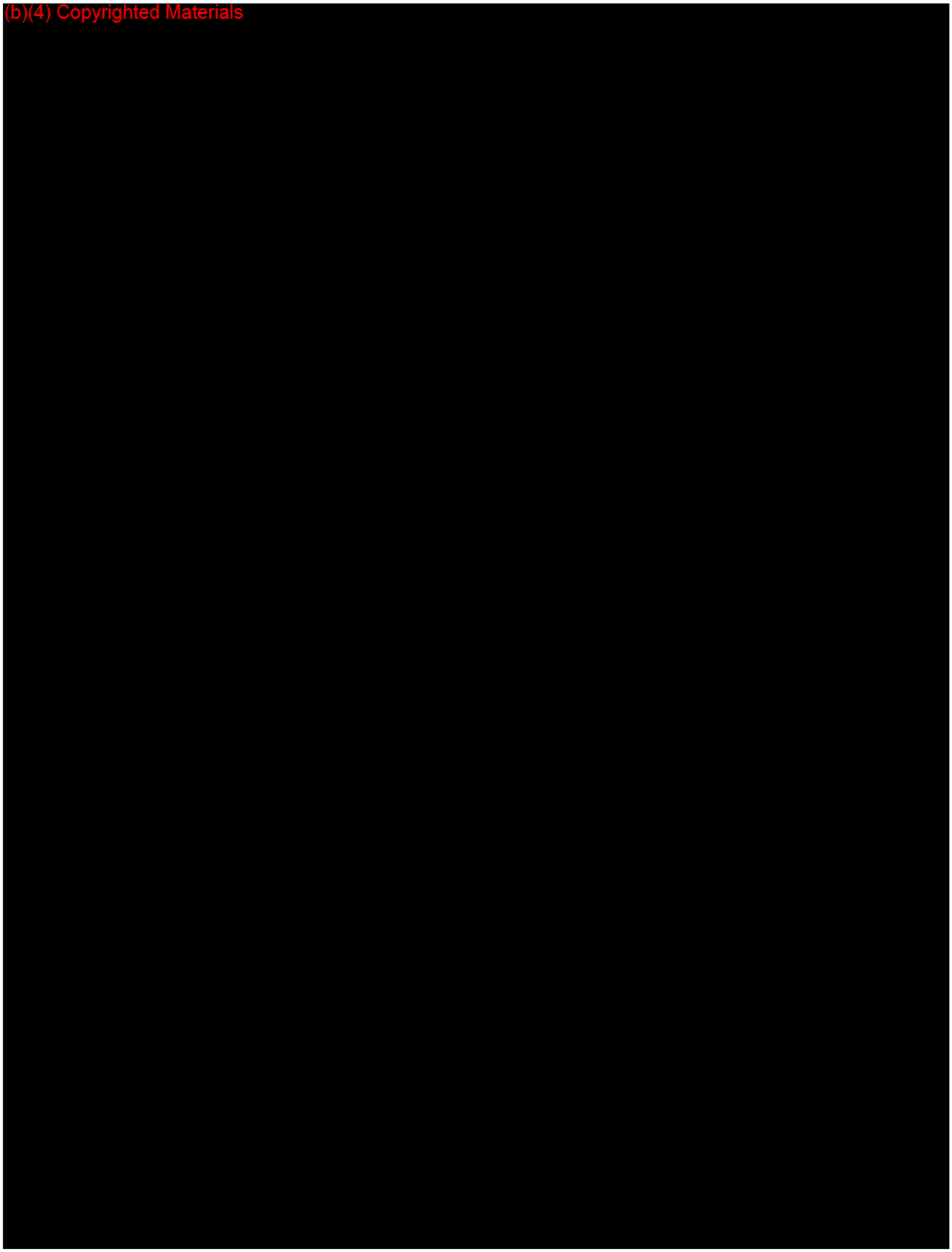

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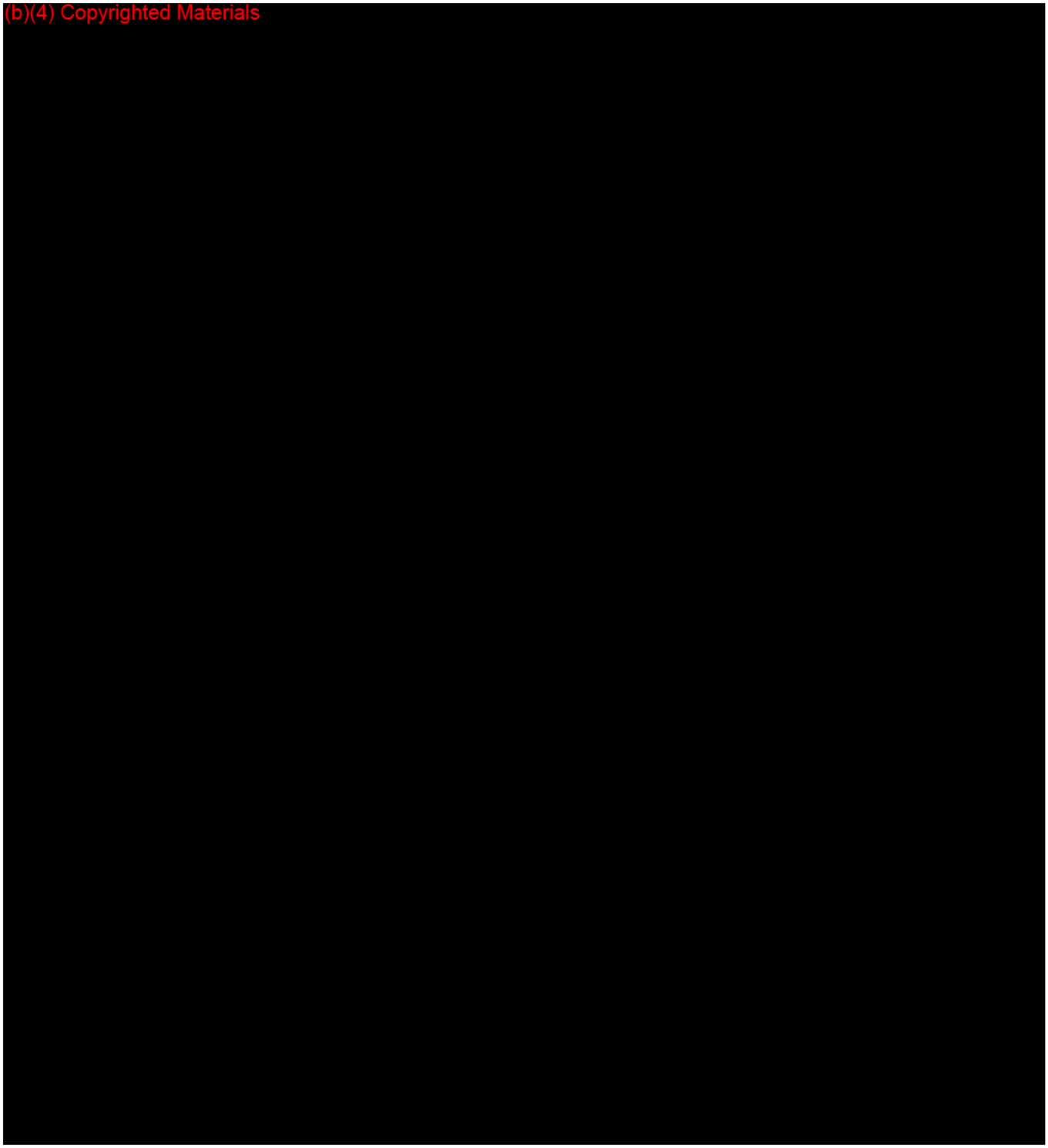

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Clinical Pharmacology

Glucarpidase (Carboxypeptidase G2) Intervention in Adult and Elderly Cancer Patients with Renal Dysfunction and Delayed Methotrexate Elimination After High-Dose Methotrexate Therapy

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Key Words. Carboxypeptidase G2 • Methotrexate • Toxicity • Kidney failure • Aged

Disclosure: S.S. has acted as a consultant for and received support from Protherics PLC. P.M. has acted as a consultant for Protherics PLC. T.A. is an employee of, owns stock in, and has received support from Protherics PLC. This study was supported in part by a research grant from Protherics PLC, Runcorn, UK.

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Identify risk factors for kidney failure associated with high-dose MTX therapy.
2. Discuss the therapeutic options in patients with delayed MTX elimination.
3. Discuss the potential risks and benefits of glucarpidase intervention.

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ABSTRACT

Objective. **Leucovorin and extracorporeal removal of methotrexate (MTX) have limited efficacy in delayed MTX elimination after high-dose methotrexate (HD-MTX) therapy. Glucarpidase (carboxypeptidase G2) cleaves MTX into nontoxic metabolites, but experience with this enzyme is limited in adult patients. We evaluated the effects of glucarpidase intervention in adult and elderly patients with delayed MTX elimination.**

Patients and Methods. **Forty-three patients (age, 18–78 years) with MTX serum concentrations (sMTX) of 1–1,187 μmol/l received glucarpidase, leucovorin rescue guided by MTX immunoassay, and standard supportive care. MTX and MTX metabolites were quantified in serum (24 patients) and urine (8 patients) by high-performance liquid chromatography. Contributory risk factors, toxicities, and survival were recorded in all patients.**

Results. **Glucarpidase was well tolerated and resulted in an immediate >97% reduction in sMTX, with a 0.2%–35% urinary recovery of the total MTX dose as inactive MTX metabolites. Forty (93%) of 43 patients**

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had normalization ($n = 25$) or improvement ($n = 15$) of their serum creatinine. Frequent grade III–IV MTX toxicities were hematological (60%) and mucositis (35%); only eight (19%) patients developed grade III–IV nephrotoxicity. Ten (23%) of 43 patients experienced fatal complications associated with HD-MTX therapy. Patients with three or more contributory risk factors for delayed MTX elimination had a significantly poorer survival than patients with fewer than three risk factors (hazard ratio, 3.64; confidence interval, 1.14–17.54).

Conclusions. **Glucarpidase is well tolerated and produces a rapid inactivation of substantial amounts of MTX. However, overall results are still unsatisfactory in adult and elderly patients, suggesting that earlier recognition of delayed MTX elimination and more rapid intervention are needed.** ***The Oncologist*** 2007;12:1299–1308

Introduction

High-dose methotrexate (HD-MTX) is frequently used in various malignancies, including acute lymphoblastic leukemia, osteosarcoma, central nervous system (CNS) lymphoma, and leptomeningeal cancer [1–4]. With leucovorin rescue and standard supportive care, HD-MTX carries a low risk for severe treatment-related toxicity. However, around 2%–10% of patients experience grade ≥2 HD-MTX–related nephrotoxicity, which could result in delayed further treatment or may occasionally be life threatening. Frequencies of grade 3 or 4 HD-MTX–related nephrotoxicity vary from only 0.6% in osteosarcoma patients to 4%–5% in mostly elderly patients with primary CNS lymphoma [5–7]. Precipitation of methotrexate (MTX) in renal tubules is thought to be the main mechanism causing HD-MTX–induced renal failure, and consequently, prolonged exposure to toxic blood MTX concentrations. Various types of renal replacement therapies have been used to enhance MTX clearance, but these require invasive access, have a limited effect in lowering blood MTX levels with rebound increase in MTX levels on termination, and are not always readily available [8].

Soon after the introduction of MTX into anticancer therapy, a loss of its biological activity was observed after contact with several bacteria. In 1967, a bacterial enzyme that cleaves glutamate from folates was identified and named carboxypeptidase G [9, 10]. Carboxypeptidase G1, isolated from *Pseudomonas stutzeri*, was successfully used to lower MTX blood levels in animal models and man, but its bacterial source was lost [11, 12]. In 1983, the gene for carboxypeptidase G2 (now termed glucarpidase), derived from *Pseudomonas* sp. strain RS-16, was cloned into *Escherichia coli*, allowing the production of sufficient amounts of this enzyme for therapeutic purposes [13, 14]. Enzymatic cleavage of glutamate from MTX by glucarpidase results in formation of the nontoxic metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) [15]. In an early study, glucarpidase, together with thymidine and leucovorin, was given to 20 patients, mainly children and young adults, who experienced HD-MTX–induced renal failure. Glucarpidase caused a pronounced and rapid reduction in circulating MTX, and only mild-to-moderate MTX toxicity occurred [16]. A subsequent study evaluated glucarpidase treatment in 65, mostly pediatric, patients with HD-MTX–induced renal failure. Following glucarpidase intervention, grade 3 or 4 MTX-related toxicities occurred in up to 40% of patients, but only four patients died as a result of HD-MTX–related myelosuppression or infectious complications [17].

To extend these observations in a larger cohort of adult patients with HD-MTX–induced renal failure, including patients of advanced age, we initiated a nationwide study in Germany. In particular, we were interested to assess the amount of enzyme-inactivated MTX by quantification of MTX metabolites in urine samples. Furthermore, we evaluated risk factors contributing to renal injury during HD-MTX therapy [18–21].

Patients and Methods

Eligibility and Enrolment

Patients receiving MTX at a dose of >1 g/m^2 body surface area (BSA) for a solid tumor or hematopoietic malignancy were eligible if their serum MTX concentration (sMTX) remained at >5 μmol/l at ≥42 hours after the start of MTX therapy. Patients with renal failure (serum creatinine [sCrea] >1.5× the upper limit of normal and/or oliguria) were also eligible if this occurred <42 hours after the start of MTX therapy or their sMTX remained at >1 μmol/l at ≥42 hours or >0.4 μmol/l at ≥48 hours after the start of MTX therapy. Following patient registration (24-hour service), the study drug was immediately dispatched by a courier service.

The study was approved by the ethical committee of the Charité and registered with the national regulatory authority (Bundesamt für Arzneimittel und Medizinprodukte, Bonn, Germany). Informed and written consent was always obtained according to the Declaration of Helsinki. The study protocol was offered to 110 centers collaborating

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within the German multicenter study group for treatment of adult acute lymphoblastic leukemia.

Glucarpidase Treatment and Supportive Care

Glucarpidase, manufactured by the Centre for Applied Microbiology and Research (Salisbury, UK), was provided as a lyophilisate (1,000 units per vial) [13, 14]. Following reconstitution in isotonic saline, glucarpidase was given i.v. over 5 minutes (scheduled dosage, 50 units/kg). A second glucarpidase treatment was optional in patients with sMTX >0.1 μmol/l $\geq$24 hours after the first intervention. Continuation of supportive care measures (i.v. fluid intake $\geq$3 l/m^2 BSA within 24 hours, urine pH $\geq$7, leucovorin) was recommended until sMTX was reduced to <0.1 μmol/l twice within 24 hours. Patients with sMTX $\leq$5 μmol/l received 15–75 mg/m^2 BSA of leucovorin i.v. every 6 hours, according to a previously published dosing schedule [22]. Patients with sMTX >5 μmol/l received leucovorin doses as follows: leucovorin (mg) = sMTX (μmol/l) $\times$ body weight (kg). Decisions on secondary glucarpidase treatment and leucovorin rescue were based on a readily available MTX immunoassay.

Patient Monitoring

Adverse events and toxicity were assessed daily. Serum creatinine, electrolytes, and blood cell counts were monitored daily and differential blood count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, prothrombin time, and activated partial thromboplastin time were measured thrice weekly. Information on the following risk factors, possibly contributing to HD-MTX–induced renal failure, were requested: body mass index (BMI), any comedication potentially interfering with MTX excretion or with known nephrotoxicity, whether the urine pH was measured as <7 at any time or the i.v. fluid intake was <3 l/m^2 BSA within 24 hours, any hepatic dysfunction at baseline, presence of a third space, urinary tract obstruction, or baseline renal dysfunction. Clinical, routine laboratory, and toxicity data [23] were recorded in standardized case report forms. sMTX was determined locally by a commercially available fluorescence polarization immunoassay (Abbott, Wiesbaden, Germany) [24].

Pharmacokinetic Sampling

Serum samples for high-performance liquid chromatography (HPLC) analyses were requested immediately before and 15, 60, and 240 minutes after glucarpidase treatment (either primary or secondary), and thereafter once daily. To inactivate glucarpidase, tubes prefilled with 1 N hydrochloric acid were provided. Blood samples were immediately put on ice and, after centrifugation, serum was transferred into the prepared tubes to give a final concentration of 0.1 N hydrochloric acid.

In a subset of patients, whole urine sampling was started after registration until glucarpidase treatment and continued thereafter until sMTX declined to <0.1 μmol/l. The total urine volume of each container and time intervals of urine sampling were recorded. Specimens were drawn from each urine container after gentle mixing. All samples were stored at least at -20°C until HPLC analysis.

HPLC Assay of MTX and MTX Metabolites in Serum and Urine

Concentrations of MTX, 7-OH-methotrexate (OH-MTX), DAMPA, and OH-DAMPA were determined by isocratic reversed-phase HPLC with UV detection at 315 nm. Serum samples were deproteinized with perchloric acid. Urine samples were diluted with buffer. Separation was performed on a C18 column filled with spherical beads (Nucleosil™; Macherey & Nagel, Düren, Germany) or a monolithic rod (Chromolith Speed Rod™; E. Merck, Darmstadt, Germany). Validation of the method yielded a limit of quantitation of 0.08 μmol/l for serum, a precision between series of 4.3%–6.9% (coefficient of variation), and recovery rates of 96%–105%. Commercially available standards for MTX, OH-MTX, and DAMPA were used (Sigma-Aldrich, Deisenhofen, Germany), whereas OH-DAMPA was produced by enzymatic cleavage of OH-MTX with glucarpidase and identified in chromatograms. Approximate concentrations of OH-DAMPA were estimated by reference to the calibration of OH-MTX.

The amounts of recovered MTX and MTX metabolites within urine sampling periods were calculated as follows: urine concentration (mol/l) $\times$ urine volume (l) = amount of excreted substance (mol). The percentages of molar quantities of the total MTX dose administered, recovered from urine samples as MTX and MTX metabolites, were calculated.

Immunogenicity Testing

Antiglucarpidase IgG antibody responses were assessed using an enzyme-linked immunosorbent assay. Briefly, microtiter plate wells were coated with glucarpidase prior to incubation with serum samples. Biotinylated protein G (Biogenesis, Poole, UK) and streptavidin horseradish peroxidase (DAKO, Ely, UK) were then added, followed by tetramethylbenzidine as a chromogenic reagent (Europa Bioproducts, Wicken, UK). A positive signal was indicated by an increase in optical absorbance, measured at 450 nm, above the limit of detection (LOD). The LOD was defined as the mean plus two standard deviations of absorbance values from control samples. Serum from subjects who had not

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been exposed to glucarpidase served as negative controls, whereas a rabbit antiglucarpidase antibody (CytImmune Sciences Inc., College Park, MD) was used as a positive control.

Serum samples that tested positive for antiglucarpidase antibodies were subsequently assayed to determine the effects on glucarpidase activity. The activity of glucarpidase, added to test samples, was determined using an enzyme-substrate spectrophotometric method, where cleavage of the substrate, MTX (Sigma-Aldrich, Gillingham, UK), was recorded by UV absorbance at 320 nm against time (Ultrospec 4000; Amersham Biosciences, Amersham, UK). By comparing the gradients of reactions, the enzyme activity in test versus control samples was calculated. A significant reduction in enzyme activity was considered when the glucarpidase activity was reduced to <80%.

Statistical Methods

Because of skewed distributions of continuous variables, medians and ranges are given for descriptive analyses. Survival data were analyzed using the Cox proportional hazard model. Patients whose death was unrelated to HD-MTX were censored at their last follow-up. The level of significance was 0.05 (two-sided). SPSS® for Windows™, release 11.5 (SPSS Inc., Chicago, IL), was used for statistical analyses.

Results

Baseline and Treatment Characteristics

Forty-three patients with acute lymphoblastic leukemia ($n = 13$), lymphoma with ($n = 16$) or without ($n = 12$) CNS involvement, germ cell tumor ($n = 1$), or osteosarcoma ($n = 1$) were registered (Table 1). Treatment with HD-MTX was given within ($n = 26$) or outside ($n = 17$) clinical trials. Treatment centers were academic medical centers ($n = 29$), large community hospitals with an inpatient capacity ≥700 ($n = 11$), or small community hospitals ($n = 3$). HD-MTX infusion times were 3–6 hours ($n = 20$) and 23–26 hours ($n = 23$). At registration, sMTX was in the range of 1–1,187 μmol/l (median, 10.5 μmol/l). The majority of patients (40/43) already had elevated sCrea and three patients developed oliguria (Table 1). The median time from the start of HD-MTX infusion to first glucarpidase treatment was 56 hours. At first treatment, the majority of patients ($n = 32$) received a glucarpidase dose close to the protocol recommendation of 50 units/kg (47–58 units/kg). The remaining 11 patients received lower doses (10–31 units/kg) because of a transient shortage of supply during the early part of the study. Except for an allergic skin reaction with pruritus (grade III), which first occurred 3 days after treatment in one patient, and fever (grade II) in another patient, glucarpidase was tolerated without side effects. Both events resolved without sequelae. A second dose of glucarpidase was administered to three patients 24–41 hours after the first dose; no side effects were reported after the second dose (Table 1). In all patients, total doses of leucovorin were 180–35,820 mg (median, 3,380 mg). At 42 hours after the start of the HD-MTX infusion, ratios of the leucovorin dose administered to the scheduled leucovorin dose varied in the range of 1%–215% (median, 27%).

Table 1. Patient and treatment characteristics

Characteristic	Value
n of patients	43
Age, years, median (range)	54 (18–78)
Sex, male/female	32/11
Underlying disease	
Acute lymphoblastic leukemia	13
Lymphoma	12
CNS lymphoma	16
Germ cell tumor	1
Osteosarcoma	1
Disease status[a]	
First diagnosis	29
Relapse/refractory	8/5
High-dose MTX regimen	
1.2–12.1 g/m^2 in 3–6 hours	20
0.9–5 g/m^2 in 23–26 hours	23
Combination chemotherapy	24
Elevated baseline serum creatinine[b]	40
Serum MTX at registration, μmol/l, median (range)	10.5 (1–1,187)
First glucarpidase treatment	
Dosage, units/kg, median (range)	50 (10–58)
Time point,[c] hours, median (range)	56 (27–176)
Second glucarpidase treatment	3
Dosage, units/kg, median (range)	49 (41–53)
Time point,[c] hours, median (range)	74 (72–102)

Number of patients is given if not stated otherwise.
[a]One patient with missing data.
[b]Prior first glucarpidase treatment.
[c]After start of high-dose MTX.
Abbreviations: CNS, central nervous system; MTX, methotrexate.

Risk Factors for HD-MTX–Induced Renal Failure

Twenty-six (60%) patients who were overweight (BMI, 25–43 kg/m^2) received 1.5–8 g/m^2 (median, 2.9 g/m^2) MTX (Table 2). Twenty-one (49%) patients had comedications with nephrotoxic potential ($n = 7$) or known interfer-

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Table 2. Risk factors contributing to delayed methotrexate (MTX) clearance in 43 patients treated with glucarpidase

Type of risk factor	Patients with risk factor, *n* (%)
Body mass index ≥25 kg/m^2	26 (60)
Relevant comedication[a]	21 (49)
Urine pH < 7[b]	15 (35)
i.v. fluid intake <3 l/m^2/24 hours[c]	10 (23)
Hepatic dysfunction at baseline[d]	10 (23)
Third space[e]	5 (12)
Renal insufficiency prior high-dose MTX	3 (7)
Diarrhea	2 (5)

[a]Salicylates, nonsteroidal anti-inflammatory drugs, beta-lactam antibiotics, sulfonamides, aminoglycosides. [b,c]Missing data in four and two patients, respectively. [d]Liver infiltration by underlying malignant disease (n = 3), liver toxicity resulting from previous chemotherapy (n = 3), alcohol abuse (n = 1), unknown (n = 3). [e]Pleural effusions (n = 5).

ence with renal MTX elimination: salicylates (n = 5), nonsteroidal anti-inflammatory drugs (n = 6), beta-lactam antibiotics (n = 8), sulfonamides (n = 7), and aminoglycosides (n = 1). A urine pH <7 was recorded in 15 (35%) patients. Other risk factors were reported in 5%–23% of patients (Table 2). Two patients (5%) had diarrhea and none of the patients had urinary tract obstruction. Only four (9%) patients had no identifiable risk factor.

Effects of Glucarpidase on Serum Levels of MTX and MTX Metabolites

In this study of a rare medical emergency, serum samples from 24 (56%) of 43 patients were available for HPLC analyses. In the remaining 19 patients, serum samples were not provided for a variety of reasons (e.g., glucarpidase treatment outside usual working hours with lack of appropriate laboratory facilities). In 24 patients with available samples, sMTX decreased markedly within 7–50 minutes (median, 15 minutes) after intervention with glucarpidase from a median of 5.1 μmol/l (range, 0.4–165.9 μmol/l) to a median of <0.1 μmol/l (range, <0.1–1 μmol/l), which corresponds to a >97% reduction (Fig. 1). Simultaneously, DAMPA serum levels increased, with a median DAMPA increase to MTX decrease ratio of 70% (data not shown).

Serial serum samples were assayed to detect rebound increases in sMTX during follow-up (median, 105 hours). In all 24 evaluable patients, the median sMTX increased from a nadir of <0.1 μmol/l (range, <0.1–1 μmol/l) to 0.24 μmol/l (range, <0.1–14.8 μmol/l) during follow-up, which corresponds to a median sustained reduction in sMTX of 97% (range, 18%–99%). In 23 patients, sMTX rebound increases did not exceed 1.2 μmol/l. The remaining patient had a sMTX of 165.9 μmol/l, and showed a rapid decline to 1 μmol/l within 15 minutes after glucarpidase treatment with a subsequent maximum increase to 14.8 μmol/l at 85 hours after intervention (Fig. 2A). Nine of 11 patients with early (<56 hours) glucarpidase intervention, compared with 10 of

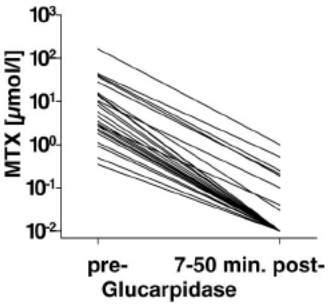

Figure 1. Immediate reduction in serum methotrexate (MTX) levels in 24 patients treated with glucarpidase.

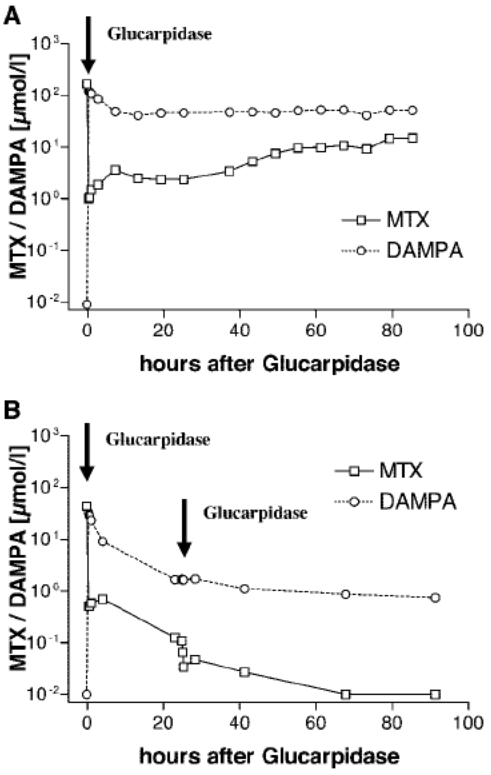

Figure 2. Serum MTX and DAMPA levels following glucarpidase treatment. **(A):** Patient with baseline serum MTX level of 165.9 μmol/l and low urine output (0.1 l/hour). **(B):** Patient with baseline serum MTX level of 43.5 μmol/l and high urine output (0.31 l/hour).

Abbreviations: DAMPA, 2,4-diamino-N^{10}-methylpteroic acid; MTX, methotrexate.

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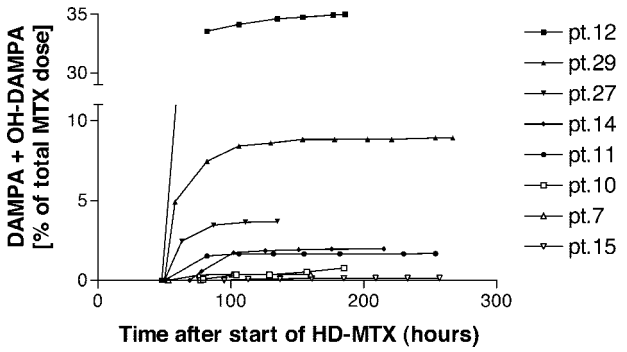

Figure 3. Recovery of the inactive MTX metabolites DAMPA and OH-DAMPA from urine samples in eight patients following glucarpidase treatment.

Abbreviations: DAMPA, 2,4-diamino-N^{10}-methylpteroic acid; MTX, methotrexate.

13 patients with late (≥56 hours) intervention, had sustained ≥90% reductions in sMTX (not significant).

Repeat glucarpidase treatment during the same cycle of HD-MTX was given to three patients. In two of these patients, sMTX prior to the second intervention was greatly overestimated because of interference of DAMPA in the immunoassay (sMTX immunoassay, 4.1 and 4.1 μmol/l; sMTX HPLC, 0.13 and 0.08 μmol/l). In these two patients, sMTX further decreased slightly to 0.11 and <0.08 μmol/l, respectively, after repeat intervention (Fig. 2B). The remaining patient had a sMTX of 0.5 μmol/l (HPLC) and a low concentration of DAMPA (0.01 μmol/l) prior to repeat intervention. In this patient, sMTX declined from 0.5 μmol/l to undetectable after repeat intervention.

The median reduction in serum concentration of OH-MTX in 21 patients was 43% (range, 18%–99%), whereas three patients had 101%–138% increases in OH-MTX concentration within 7–15 minutes after glucarpidase administration (data not shown).

Urine Recovery of MTX Metabolites After Glucarpidase Treatment

Urinary excretion of DAMPA and OH-DAMPA was quantified in eight patients during an 85- to 217-hour follow-up. Proportions of the total MTX dose, recovered as DAMPA and OH-DAMPA, varied from 0.2%–35% (Fig. 3). In two patients, the urine recovery rates of DAMPA and OH-DAMPA were 35% and 8.9%, compared with only ≤3.7% in the other six patients. These two patients had higher pretreatment sMTX than all other patients (≥28.3 μmol/l versus ≤5.8 μmol/l), with the exception of a single patient, and received their first glucarpidase intervention early (48 and 50 hours, versus ≥50 hours in all other patients after the start of HD-MTX). One patient received glucarpidase at 50 hours after the start of HD-MTX when his sMTX was 165.9 μmol/l, but his DAMPA and OH-DAMPA urine recovery was only 3.7%. This patient had an average urine output of only 0.1 l/hour and a sCrea of 310 μmol/l prior to glucarpidase, compared with a median urine output of 0.3 l/hour and a sCrea of ≤238 μmol/l in all other patients. Furthermore, this particular patient was treated with a single dose of only 11 units/kg glucarpidase, compared with a median glucarpidase dose of 50 units/kg in all other patients. Finally, the serum DAMPA concentrations in this patient remained >40 μmol/l (Fig. 2A), whereas all other patients had steadily declining serum DAMPA concentrations (Fig. 2B).

Immunogenicity of Glucarpidase

Serum samples for immunogenicity testing, obtained 7–41 days after glucarpidase treatment, were available from seven patients. In three of these seven patients, antiglucarpidase antibodies were first detected 7–17 days after glucarpidase administration, whereas the remaining four patients did not show antibody responses during 7–41 days of follow-up. All positive samples were additionally tested for their effect on glucarpidase activity. Only one of four antibody-positive samples showed a slight (23%) reduction in glucarpidase activity.

Recovery from Renal Failure, MTX Toxicity, and Outcome

The median peak sCrea in all 43 patients was 232 μmol/l. In 40 (93%) of 43 patients, the sCrea remained normal ($n = 1$), returned to normal ($n = 24$), or improved ($n = 15$) after glucarpidase intervention. Three (7%) of 43 patients showed an increasing sCrea during follow-up. In nine and 16 patients, the sCrea returned to baseline values and <1.5× the baseline level within a median of 28 days and 19 days after the start of HD-MTX, respectively. In 26 and 19 patients, the sCrea remained above these thresholds within median observation periods after the start of HD-MTX of 36 days and 12 days (eight patients with missing data), respectively. Five patients received hemodialysis ($n = 3$), hemofiltration ($n = 3$), or hemoperfusion ($n = 1$) for 2–13 days, although no absolute necessity for this was reported for four of them. The remaining patient had a decline in sCrea after glucarpidase administration, but subsequently required hemofiltration as a result of severe infection with renal failure.

Renal toxicity of any grade was the most frequently reported MTX-related toxicity (98%), but only eight (19%) patients developed grade III–IV renal toxicity (Table 3). Among other grade III–IV toxicities, hematotoxicity (60%) and mucositis (35%) were the most frequently reported. Grade IV neurotoxicity occurred in two patients with acute lymphoblastic leukemia who concurrently received a single intrathecal MTX administration (15 mg): severe depression

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Table 3. Toxicities occurring after high-dose methotrexate in 43 patients treated with glucarpidase for delayed methotrexate elimination

	Grade 0	Grade I–II	Grade III–IV
Hematological	6	8	26 (60%)
Mucositis	22	4	15 (35%)
Renal	1	34	8 (19%)
Liver	16	17	7 (16%)
Central nervous system	32	2	6 (14%)
Skin	34	6	1 (2%)
Gastrointestinal	40	3	–

Toxicity was graded according to the World Health Organization toxicity scale [23].
Missing data in three (hematological), two (mucositis), three (liver), three (central nervous system), and two (skin) patients.

with hallucinations ($n = 1$) and fatal progressive cerebral coma ($n = 1$). There was no evidence of an accidental intrathecal MTX overdose in either patient.

Ten (23%) of 43 patients died as a result of complications associated with HD-MTX: infection ($n = 7$), uremia and seizures ($n = 1$), MTX neurotoxicity ($n = 1$), and peritonitis with multiorgan failure ($n = 1$). All seven patients with fatal infectious complications had grade IV neutropenia, three of these seven patients received other chemotherapy agents in addition to HD-MTX, and death occurred in these patients 4–38 days (median, 18 days) after the start of HD-MTX. Nine (21%) patients died as a result of causes unrelated to HD-MTX: subsequent chemotherapy ($n = 5$), hematopoietic stem cell transplantation ($n = 1$), disease progression ($n = 2$), and cardiovascular event ($n = 1$).

Statistical analyses revealed no significant impact of the time of glucarpidase intervention relative to the time of MTX administration, the sMTX prior to glucarpidase administration, whether there was a sustained reduction in sMTX, the glucarpidase dose, or age on toxicities and survival. Similar analyses in subsets of patients treated with HD-MTX alone or in combination with other cytostatic drugs did not show any significant impact on toxicities and survival. In addition, the total leucovorin dose or the ratio of the leucovorin dose administered to the leucovorin dose scheduled according to sMTX at 42 hours did not correlate with toxicities and survival, except for a trend toward less severe mucositis in patients with high total leucovorin doses. However, survival in patients with three or more contributory risk factors for delayed elimination of MTX was significantly less than that of patients with fewer than three risk factors (hazard ratio, 3.64; confidence interval, 1.14–17.54; $p = .03$).

Discussion

HD-MTX–induced renal failure with persistence of toxic blood MTX levels is a rare but life-threatening complication that occurs more frequently in adults, particularly those with advanced age and CNS lymphoma [5–7]. Hemodialysis, hemoperfusion, or a combination of the two in this condition have been repeatedly investigated, but these invasive procedures require prolonged application, might add treatment-related morbidity, and result in a limited decline in blood MTX levels with frequent rebound increases [8].

In contrast, glucarpidase is well tolerated, easy to administer, and causes rapid, pronounced, and, in the majority of patients, sustained reductions in circulating MTX, as demonstrated by this study and previous trials [16, 17]. MTX is modified intracellularly by sequential addition of glutamate molecules. These MTX polyglutamates are retained within cells and are the main mediators of MTX cytotoxicity through inhibition of various folate-dependent enzymes, including dihydrofolate reductase [25]. Thus, leucovorin rescue is still required after glucarpidase intervention as glucarpidase does not hydrolyze intracellular MTX. However, removal of MTX from the circulation is expected to affect tissue levels indirectly, by preventing further MTX uptake. Leucovorin is also a substrate for glucarpidase, but with lower affinity for the enzyme than MTX, and there is a risk that interaction of glucarpidase with leucovorin may decrease the efficacy of leucovorin rescue [14].

In two patients with repeat intervention, glucarpidase showed diminished efficacy, which was likely caused by the presence of the metabolite DAMPA. Also, an immune response to glucarpidase could potentially cause a reduction in enzyme activity or adverse effects upon repeat administration. We found antiglucarpidase antibodies in three of seven study patients, but only one of four antibody-positive samples showed a slight (23%) reduction in glucarpidase activity in vitro. More data on the immunogenicity of glucarpidase are needed before considering repeat use of this enzyme.

According to a previously used definition of renal recovery (sCrea $<1.5\times$ baseline), 16 patients recovered within a median of 19 days, and 19 patients still had a sCrea above this threshold within a median observation period of 12 days [6]. This suggests that the renal recovery in this study was somewhat slower than in patients treated with supportive care measures, including extracorporeal removal of MTX but not glucarpidase, whose reported median time to renal recovery was 16 days [6]. The severity of

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renal damage in this study, as indicated by the median peak creatinine level, was, however, higher (232 μmol/l = 3 mg/dl) than that of previously evaluated patients treated with supportive care not including glucarpidase or dialysis-based interventions (2.1 mg/dl), but was less than in patients who received extracorporeal removal of MTX (3.9 mg/dl) [6]. Remarkably, some patients in this study received nephrotoxic agents in addition to HD-MTX, which may have contributed in part to delays in renal recovery. It remains unclear whether DAMPA, which is regarded as virtually inert but around 10-fold less soluble than MTX, might have caused delays in renal recovery. DAMPA is cleared by the kidneys and metabolized into OH-DAMPA and DAMPA glucuronides [15, 16]. It is unknown whether variations in DAMPA metabolism have a clinical impact, requiring more refined studies with superior analytical techniques. In patients with normal renal function, around 80% of the MTX dose is excreted renally within 24 hours [26]. It is noteworthy that, in this study, up to 35% of the total MTX dose was recovered as inactive metabolites from the urine later than 48 hours after the start of the MTX infusion.

We observed a high frequency of grade III–IV MTX-related toxicities, which is not unexpected for patients with prolonged MTX exposure. In the two major previous trials evaluating glucarpidase, frequencies of fatal HD-MTX–associated complications were <7%. In contrast, we observed a strikingly high (23%) MTX-related fatality rate, which in part could be explained by the higher median age of our study patients compared with those of both previous trials (54 versus ≤16 years) [16, 17]. The use of HD-MTX in adult patients is associated with significant treatment-related mortality, and fatality rates of 9%–10% have been seen in trials evaluating HD-MTX in previously untreated adult patients, who had renal toxicity rates of only ≤14% [27–29]. In contrast, the patients in this study all had evidence of delayed elimination of MTX, which likely increased the risk for fatal MTX-related events. Except for scarce data from case reports and small case series, toxicity data and fatality rates in adult/elderly patients with HD-MTX–associated renal failure are largely unknown, which limits an estimate of the benefits of glucarpidase intervention in this particular patient subgroup. The survival and HD-MTX–related toxicities in this study did not correlate with age, baseline sMTX, time of intervention with glucarpidase, glucarpidase dose, degree of sustained reduction in sMTX, additional use of chemotherapy agents other than MTX, or inappropriately low leucovorin doses. Risk factors contributing to delayed MTX elimination were identified in the majority (91%) of patients, and the presence of multiple (three or more) risk factors correlated with fatal HD-MTX–associated complications. This emphasizes that patients should be carefully evaluated before HD-MTX is considered. Overweight is not an established risk factor associated with HD-MTX–induced renal failure, but there was a remarkably high number of overweight study patients. Pharmacokinetic data from an obese patient indicate increases in the volume of distribution and systemic clearance of MTX, which may compensate each other [30]. However, nephrotoxicity with delayed MTX elimination was reported in another obese patient treated with an intermediate dose of MTX [31]. Thus, dose adaptation guided by ideal body weight or a proposed score system might reduce the risk for HD-MTX–related toxicity in overweight patients [32].

Conclusion

In this study, glucarpidase intervention was safe and effectively reduced plasma MTX exposure in patients with delayed MTX elimination. Glucarpidase may occasionally result in antibody formation that has the potential to reduce enzyme activity upon repeat administration. Patients in this study still had a high frequency of severe toxicities, which reflects the high risks associated with delayed MTX elimination in adult patients. No conclusion can be drawn about the effects of glucarpidase on toxicity from this single-arm study, and further studies evaluating the potential of glucarpidase to reduce the risk for severe or fatal HD-MTX–induced toxicities in patients at risk are warranted. Currently, a more careful selection of patients considered suitable for HD-MTX and the use of optimal supportive care strategies should always be attempted.

Acknowledgments

The authors are grateful to E. Borner and H. Hartwig for skillful technical assistance. We are also indebted to N. Gökbuget and D. Hoelzer from the GMALL study center for their continuous support and to R. Melton for his efforts in the early phase of this study. Results from this study were presented in part at the 40th Annual Meeting of the American Society of Clinical Oncology, New Orleans, Louisiana, June 5–8, 2004.

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Glucarpidase (Carboxypeptidase G2) Intervention in Adult and Elderly Cancer Patients with Renal Dysfunction and Delayed Methotrexate Elimination After High-Dose Methotrexate Therapy

Stefan Schwartz, Klaus Borner, Krystina Müller, Peter Martus, Lars Fischer, Agnieszka Korfel, Timothy Auton and Eckhard Thiel

Oncologist 2007;12;1299-1308
DOI: 10.1634/theoncologist.12-11-1299

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ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1991, p. 1884–1890 Vol. 35, No. 9
0066-4804/91/091884-07$02.00/0
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Pharmacokinetics and Adverse Effects of 20-mg/kg/Day Trimethoprim and 100-mg/kg/Day Sulfamethoxazole in Healthy Adult Subjects

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Received 20 February 1991/Accepted 10 July 1991

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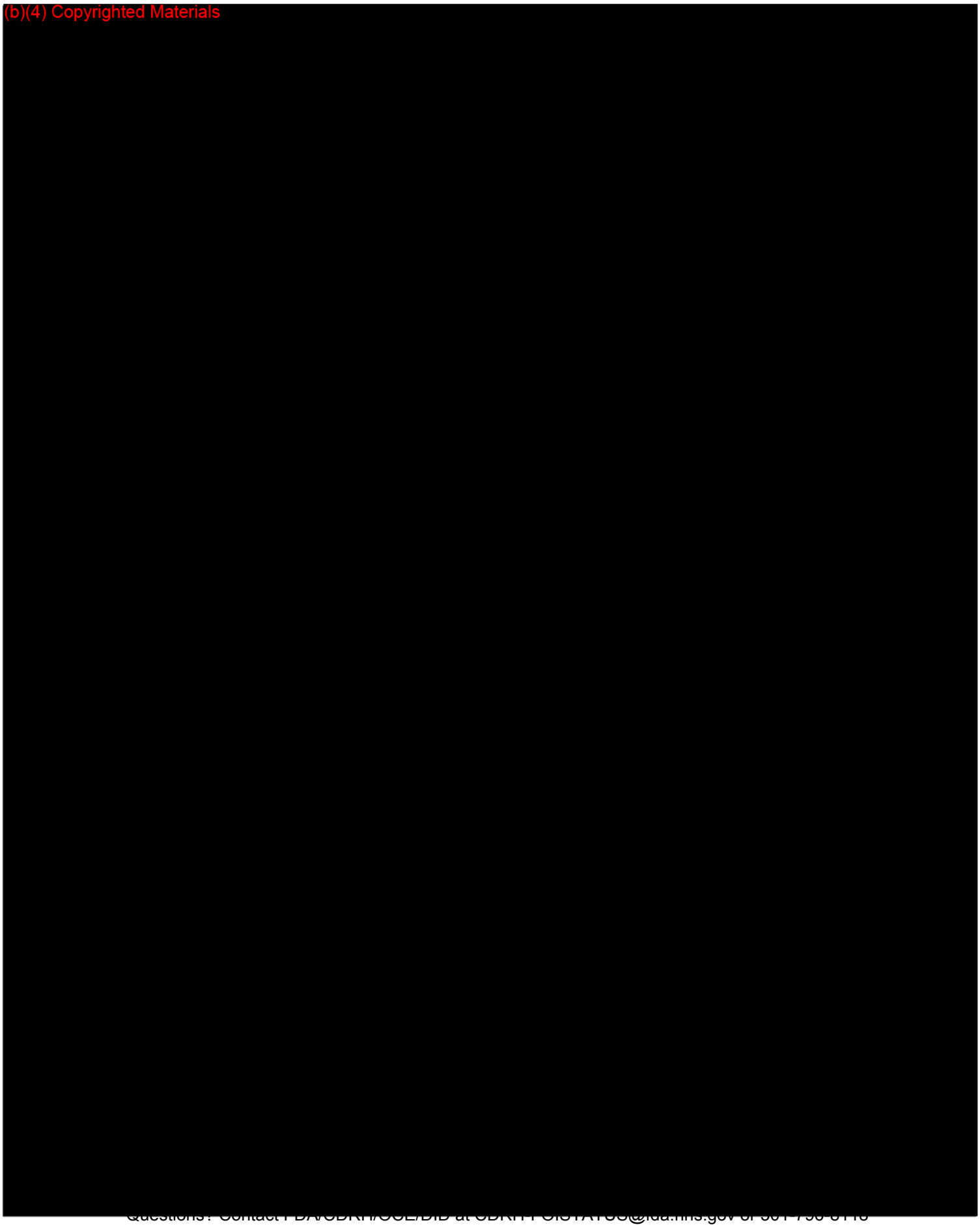

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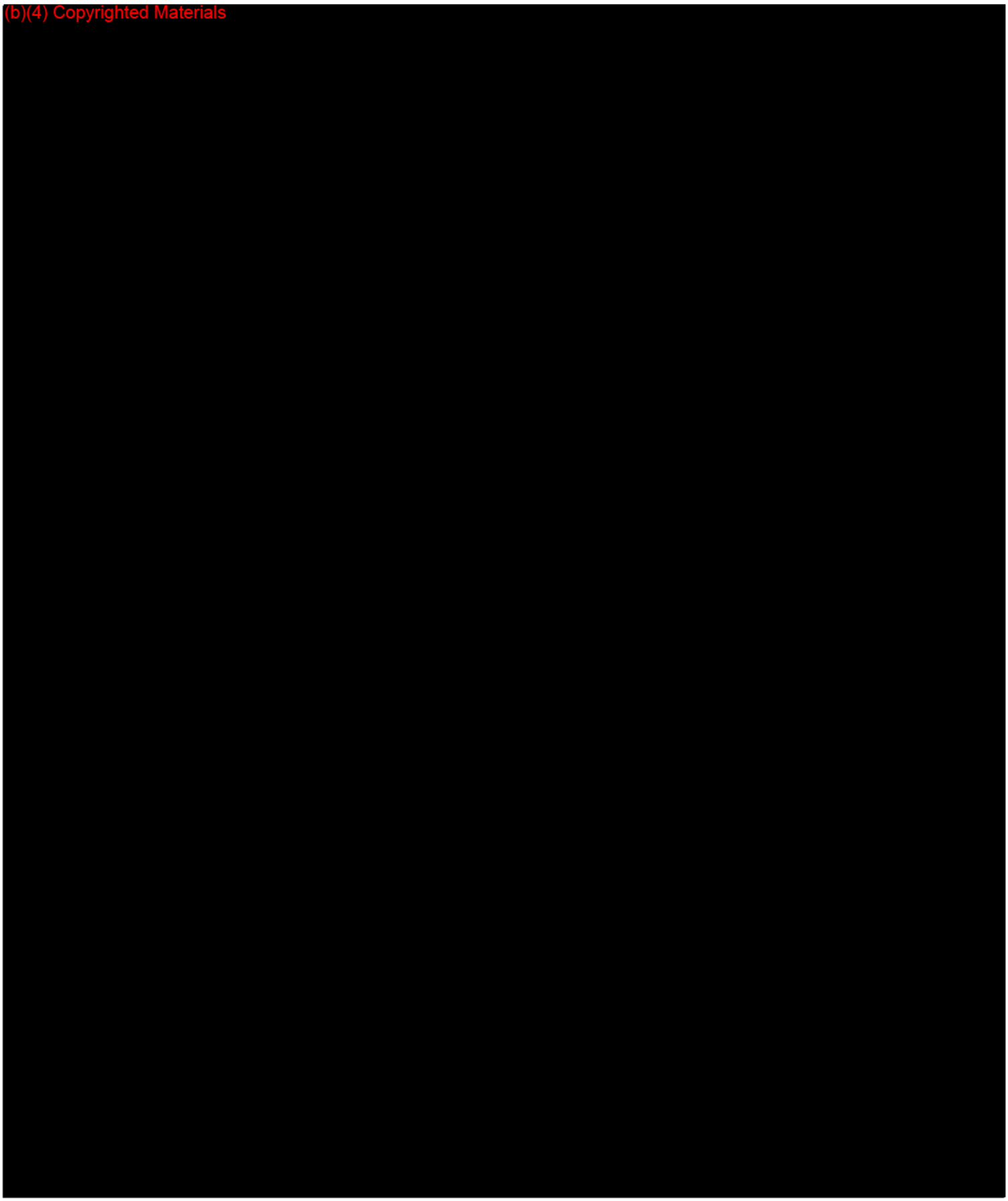

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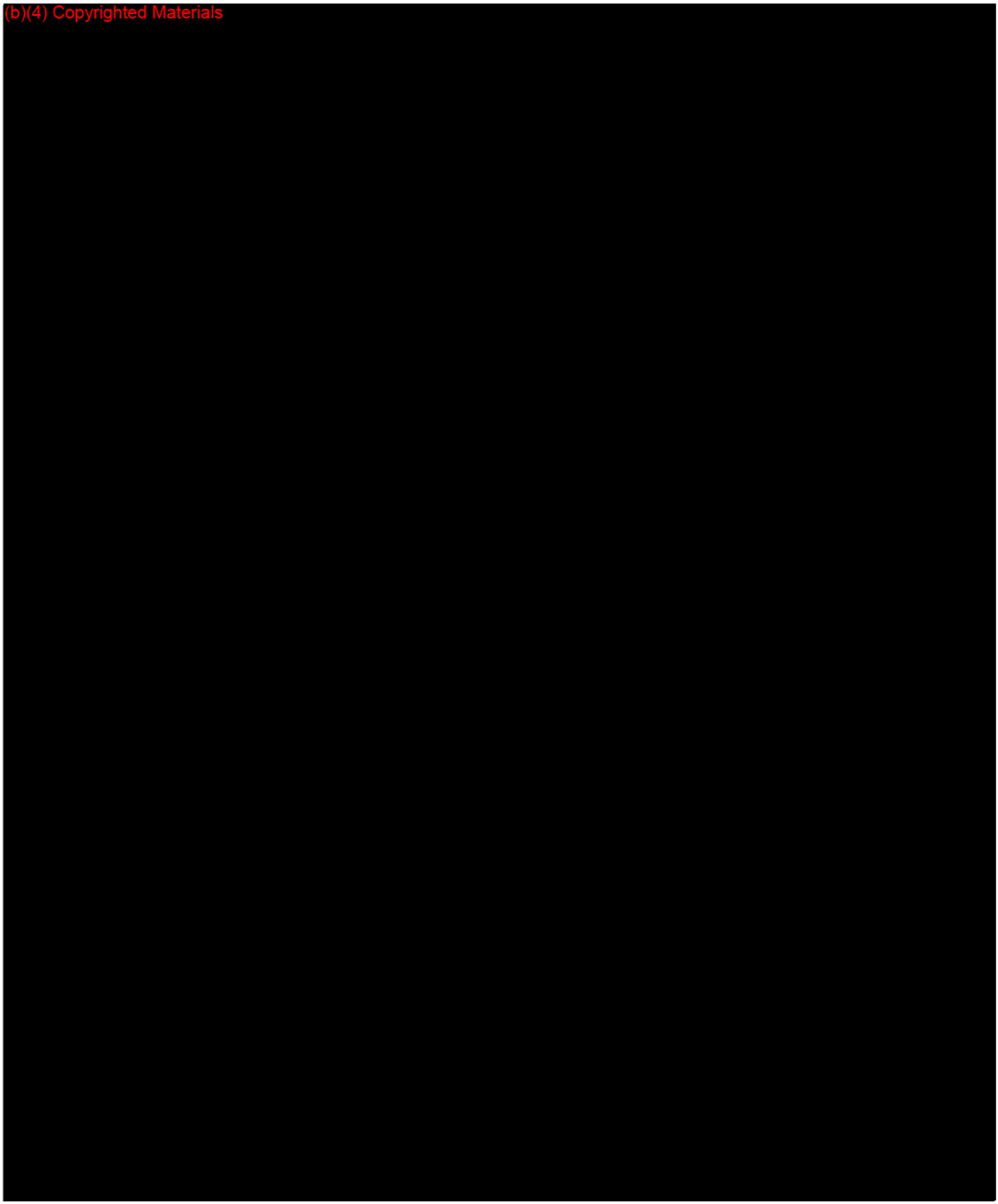

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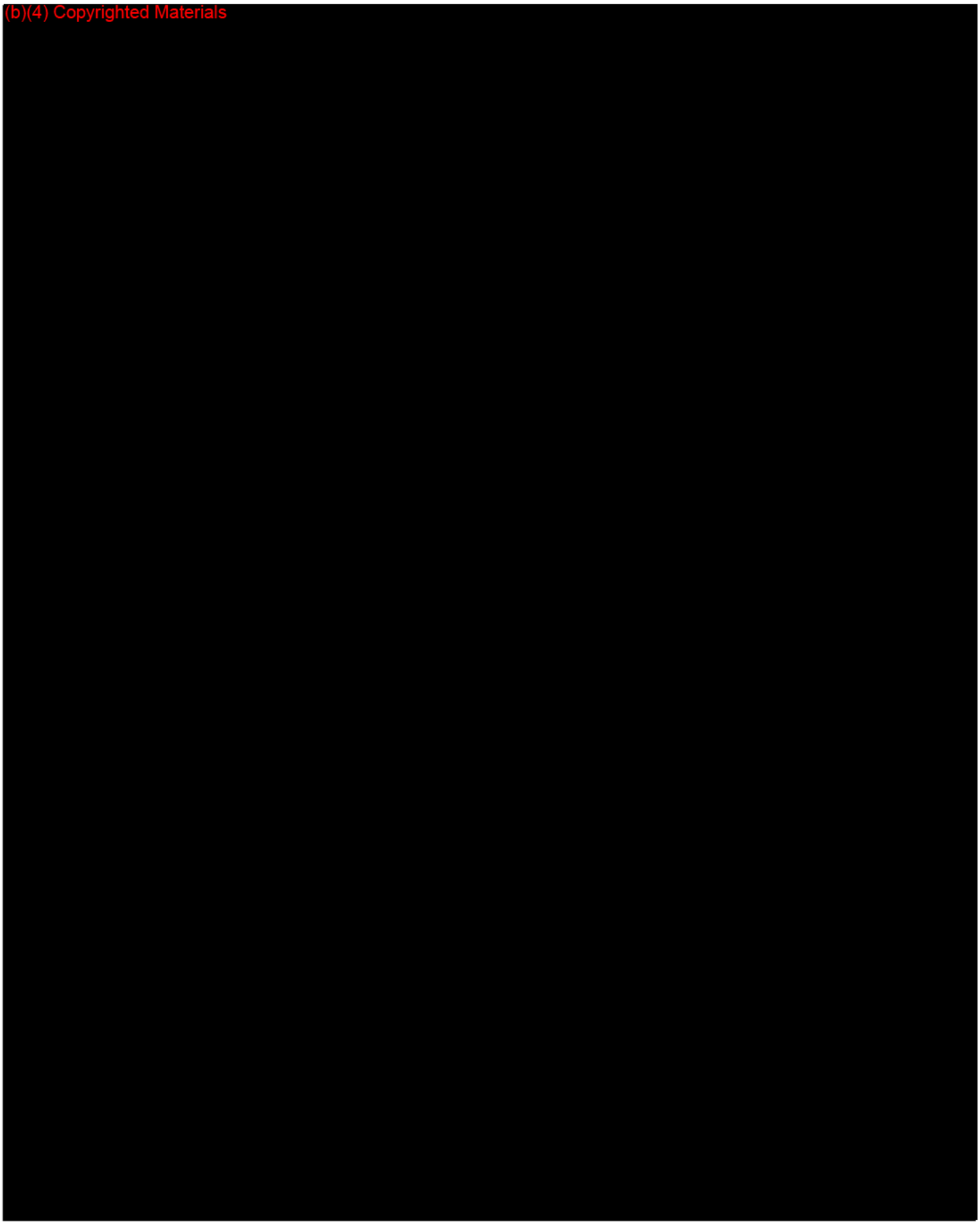

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STUDY A: LIMIT OF DETECTION/QUANTITATION (METHOTREXATE)

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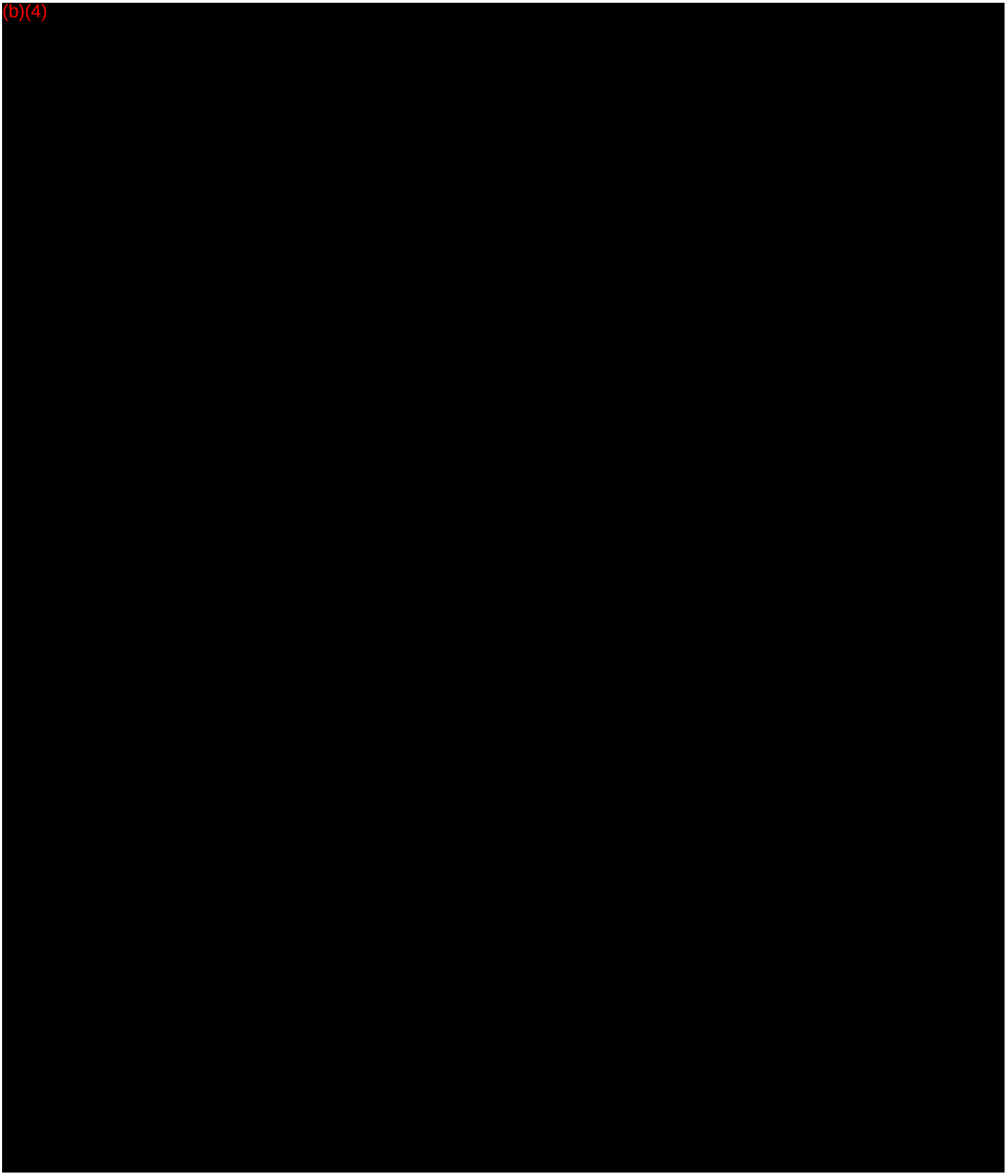

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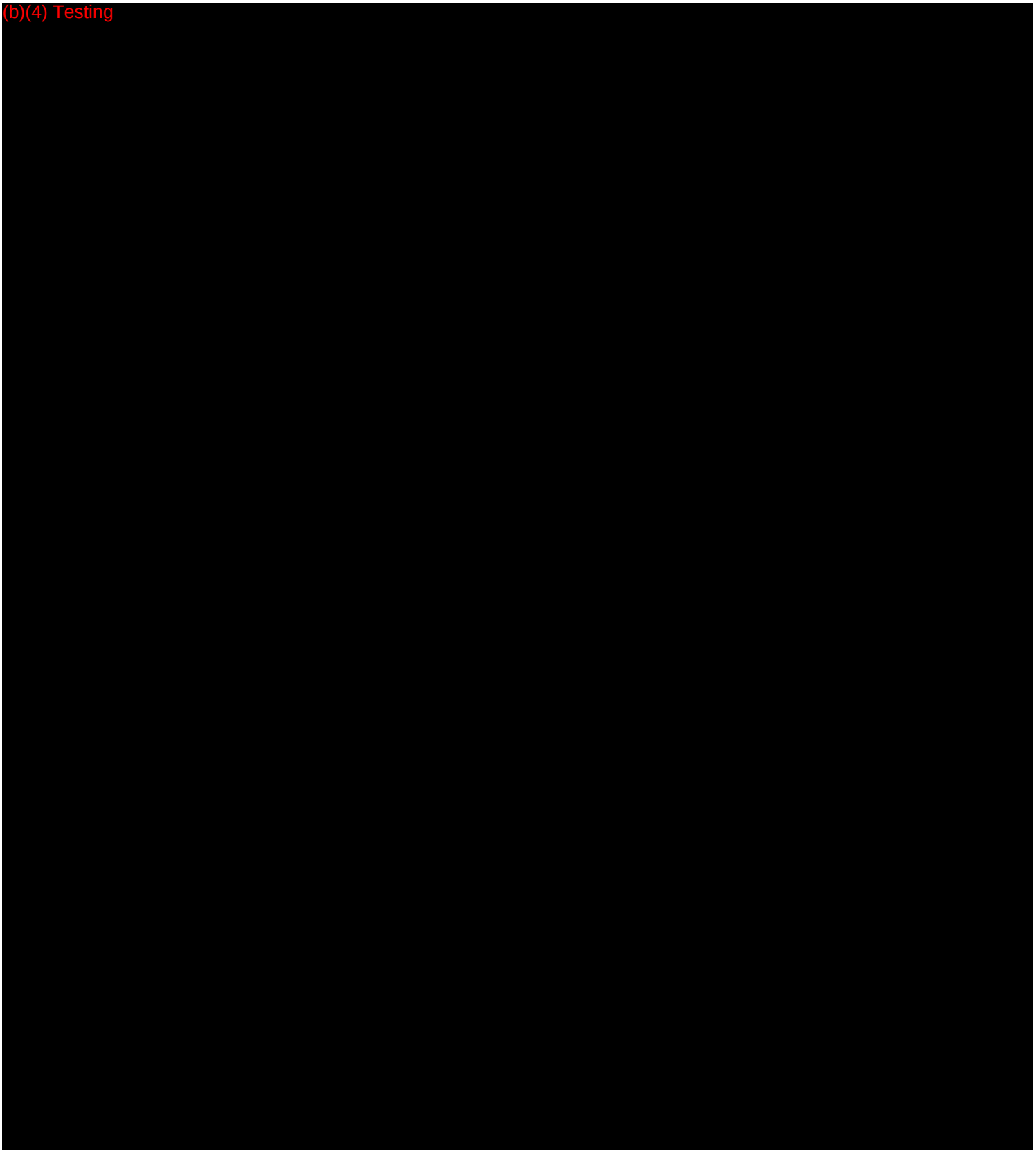

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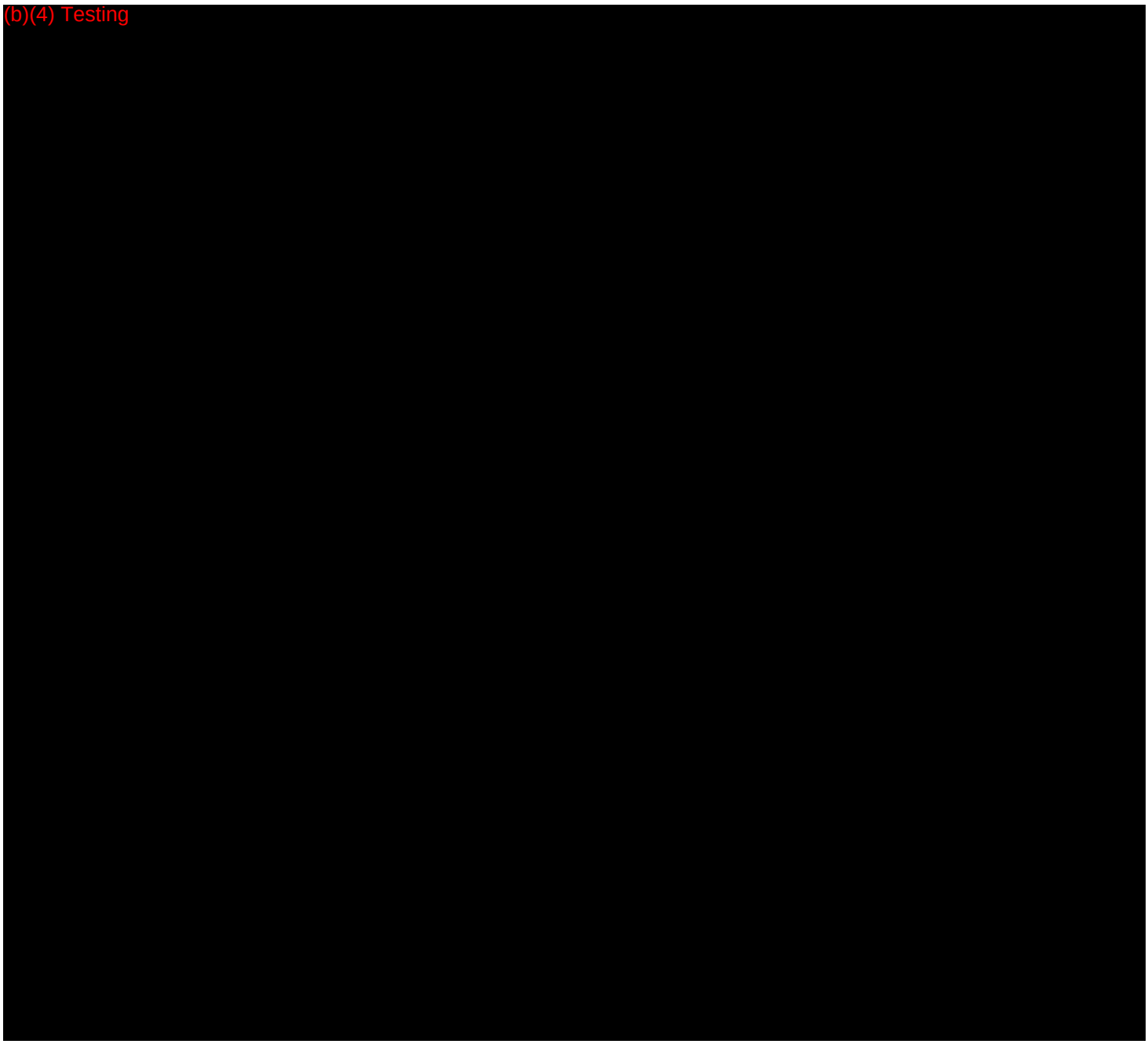

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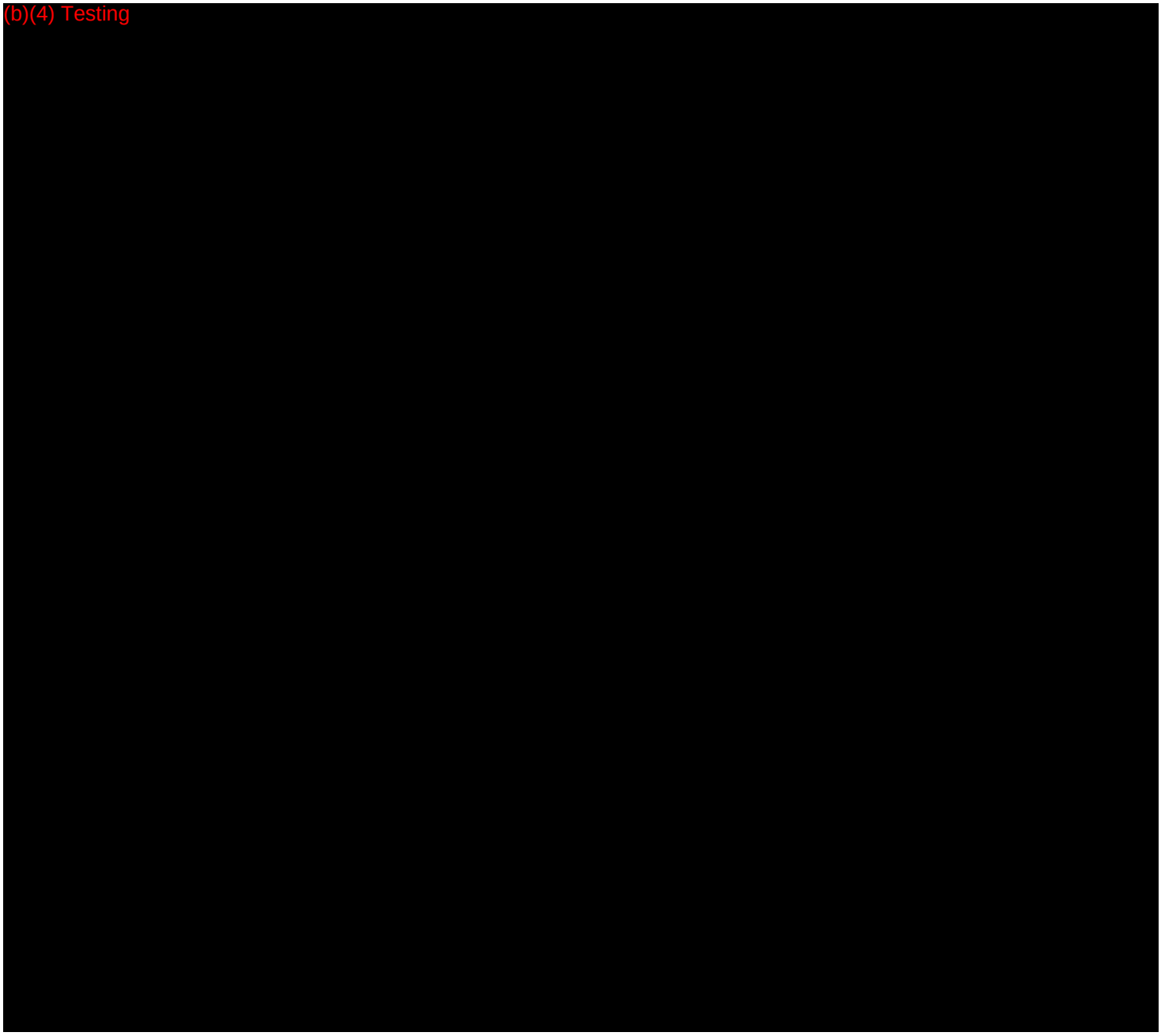

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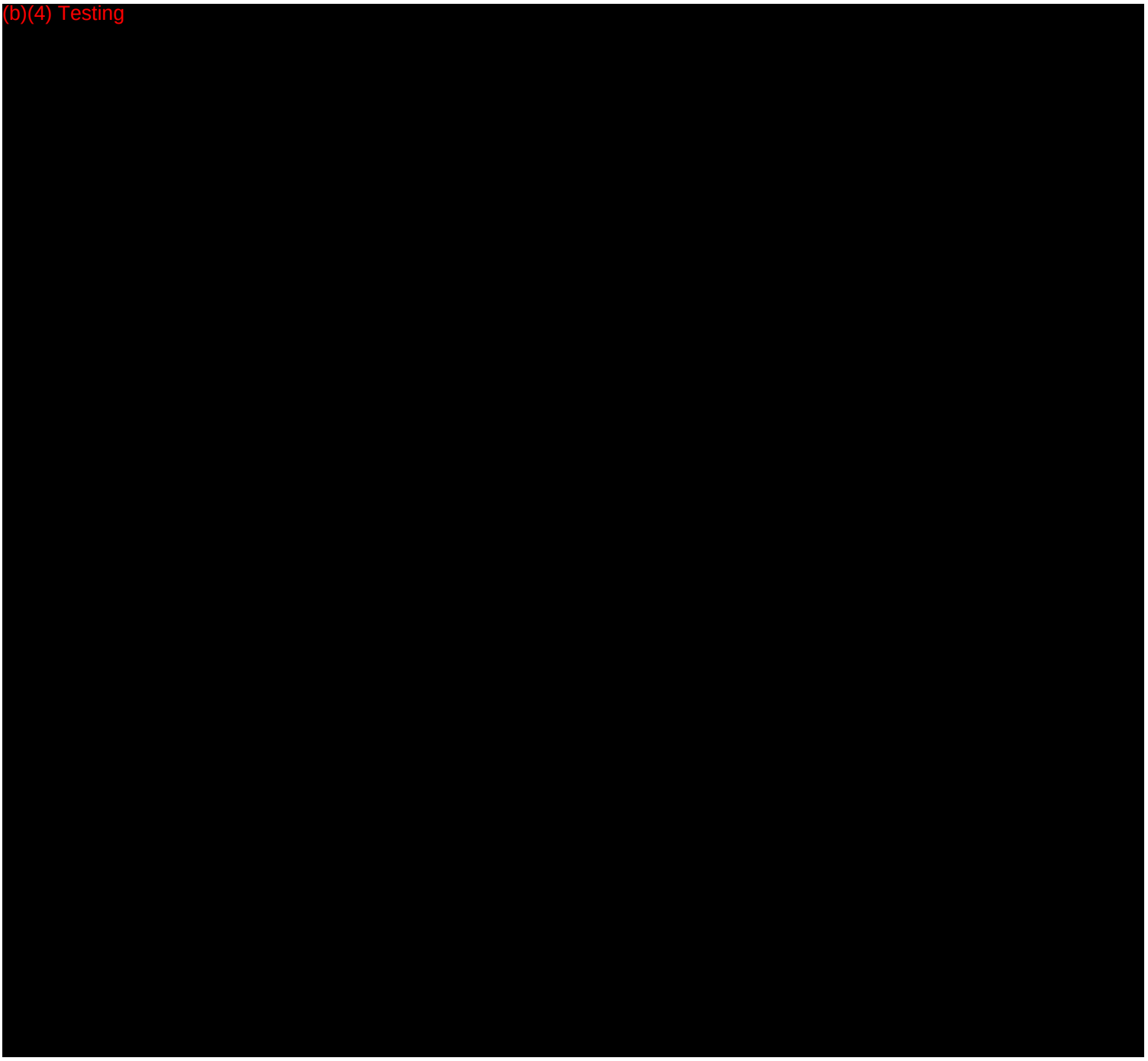

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STUDY B: RECOVERY (METHOTREXATE)

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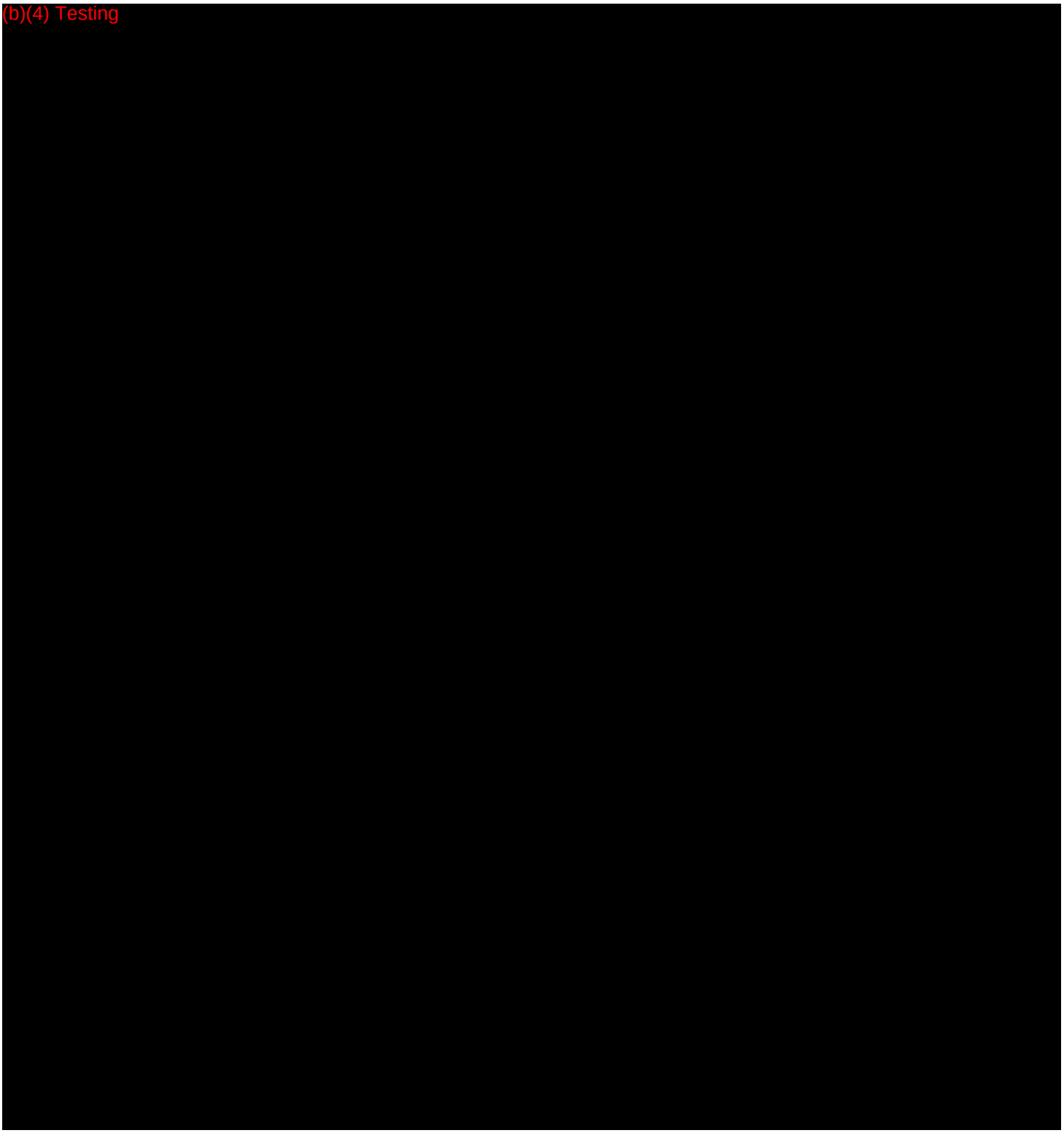

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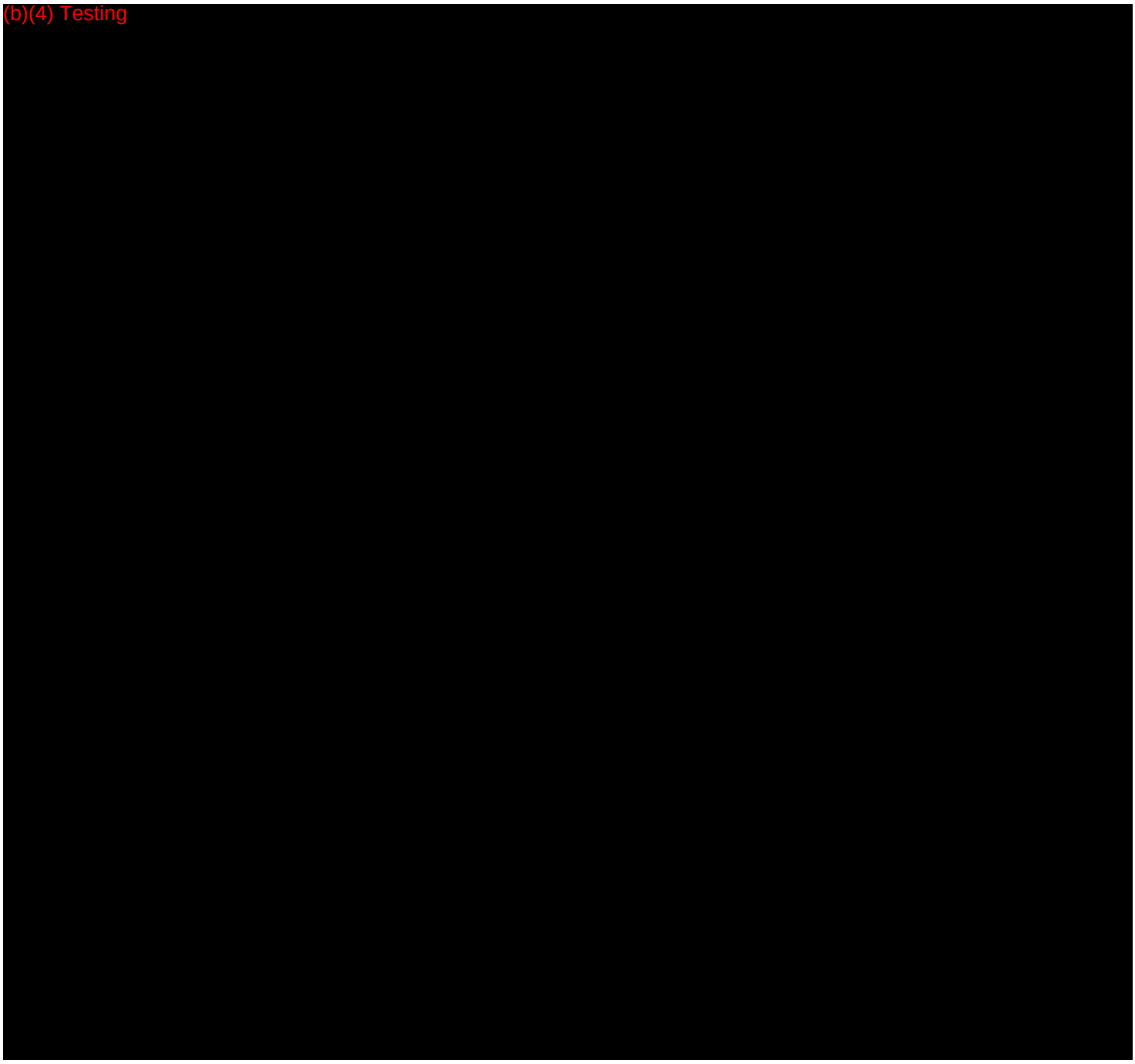

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Acute renal failure has been reported in a few patients receiving indomethacin and formulations containing Triamterene and Hydrochlorothiazide. Caution is therefore advised when administering nonsteroidal anti-inflammatory agents with Triamterene and Hydrochlorothiazide.

Potassium-sparing agents, should be used cautiously, if at all, in conjunction with Angiotensin-converting enzyme (ACE) inhibitors, due to an increased risk of hyperkalemia. Serum potassium should be monitored frequently.

Drug/Laboratory Test Interactions

Triamterene and quinidine have similar fluorescence spectra; thus, Triamterene and Hydrochlorothiazide may interfere with the measurement of quinidine.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Long term studies with triamterene/hydrochlorothiazide combination have not been conducted.

Triamterene: In studies conducted under the auspices of the National Toxicology Program (NTP), groups of rats were fed diets containing 0, 150, 300 or 600 ppm triamterene and groups of mice were fed diets containing 0, 100, 200 or 400 ppm triamterene. Male and female rats exposed to the highest tested concentration received triamterene at about 25 and 30 mg/kg/day, respectively. Male and female mice exposed to the highest tested concentration received triamterene at about 45 and 60 mg/kg/day, respectively.

There was an increased incidence of hepatocellular neoplasia (primarily adenomas) in male and female mice at the highest dosage level. These doses represent 7.5 times and 10 times the Maximum Recommended Human Dose (MRHD) of 300 mg/kg (or 6 mg/kg/day based on a 50 kg patient) for male and female mice, respectively when based on body-weight and 0.7 times and 0.9 times the MRHD when based on body-surface area. Although hepatocellular neoplasia (exclusively adenomas) in the rat study was limited to triamterene-exposed males, incidence was not dose-dependent and there was no statistically significant difference from control incidence at any dose level.

Hydrochlorothiazide: Two-year feeding studies in mice and rats, conducted under the auspices of the National Toxicology Program (NTP), treated mice and rats with doses of hydrochlorothiazide up to 600 and 100 mg/kg/day, respectively. On a body-weight basis, these doses are 600 times (in mice) and 100 times (in rats) the Maximum Recommended Human Dose (MRHD) for the hydrochlorothiazide component of Triamterene and Hydrochlorothiazide tablets (50 mg/day or 1.0 mg/kg/day based on a 50 kg patient). On the basis of body-surface area, these doses are 56 times (in mice) and 21 times (in rats) the MRHD. These studies uncovered no evidence of carcinogenic potential of hydrochlorothiazide in rats or female mice, but there was equivocal evidence of hepatocarcinogenicity in male mice.

Mutagenesis: Studies of the mutagenic potential of Triamterene and Hydrochlorothiazide combination have not been performed.

Triamterene: Triamterene was not mutagenic in bacteria (S. typhimurium strains TA 98, TA 100, TA 1535 or TA 1537) with or without metabolic activation. It did not induce chromosomal aberrations in Chinese hamster ovary (CHO) cells in vitro with or without metabolic activation, but it did induce sister chromatid exchanges in CHO cells in vitro with and without metabolic activation.

Hydrochlorothiazide: Hydrochlorothiazide was not genotoxic in in vitro assays using strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 of Salmonella typhimurium (the Ames test) in the Chinese hamster ovary (CHO) test for chromosomal aberrations, or in in vivo assays using mouse germinal cell chromosomes, Chinese hamster bone marrow chromosomes, and the Drosophila sex-linked recessive lethal trait gene. Positive test results were obtained in the in vitro CHO sister chromatid exchange (clastogenicity) test, and in the mouse hymphoma cell (mutagenicity) assays, using concentrations of hydrochlorothiazide of 43 to 1300 mcg/mL. Positive test results were also obtained in the Aspergillus nidulans nondisjunction assay using an unspecified concentration of hydrochlorothiazide.

Impairment of Fertility: Studies of the effect of triamterene/ hydrochlorothiazide combination, or of triamterene alone on animal reproductive function have not been conducted. Hydrochlorothiazide: Hydrochlorothiazide had no adverse effects on the fertility of mice and rats of either sex in studies wherein these species were exposed via their diet to doses of up to 100 and 4 mg/kg/day, respectively, prior to mating and throughout gestation. Corresponding multiples of the MRHD are 100 (mice) and 4 (rats) on the basis of body weight and 9.4 (mice) and 0.8 (rats) on the basis of body-surface area.

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Pregnancy: Category C

Teratogenic Effects

Animal reproduction studies to determine the potential for fetal harm by Triamterene and Hydrochlorothiazide tablets have not been conducted. Nevertheless, a One Generation Study in the rat approximated Triamterene and Hydrochlorothiazide's composition by using a 1:1 ratio of triamterene to hydrochlorothiazide (30:30 mg/kg/day). There was no evidence of teratogenicity at those doses that were, on a body-weight basis, 15 and 30 times, respectively, the MRHD, and, on the basis of body-surface area, 3.1 and 6.2 times, respectively, the MRHD.

The safe use of Triamterene and Hydrochlorothiazide tablets in pregnancy has not been established since there are no adequate and well-controlled studies with Triamterene and Hydrochlorothiazide tablets in pregnant women. Triamterene and Hydrochlorothiazide tablets should be used during pregnancy only if the potential benefit justifies the risk to the fetus.

Triamterene: Reproduction studies have been performed in rats at doses as high as 20 times the Maximum Recommended Human Dose (MRHD) on the basis of body-weight, and 6 times the MRHD on the basis of body-surface area without evidence of harm to the fetus due to triamterene.

Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Hydrochlorothiazide: Hydrochlorothiazide was orally administered to pregnant mice and rats during respective periods of major organogenesis at doses up to 3000 and 1000 mg/kg/day, respectively. At these doses, which are multiples of the MRHD equal to 3000 for mice and 1000 for rats, based on body-weight, and equal to 282 for mice and 206 for rats, based on body-surface area, there was no evidence of harm to the fetus. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nonteratogenic Effects

Thiazides and triamterene have been shown to cross the placental barrier and appear in cord blood. The use of thiazides and triamterene in pregnant women requires that the anticipated benefit be weighed against possible hazards to the fetus. These hazards include fetal or neonatal jaundice, pancreatitis, thrombocytopenia and possibly other adverse reactions that have occurred in the adult.

Nursing Mothers

Thiazides and triamterene in combination have not been studied in nursing mothers. Triamterene appears in animal milk and this may occur in humans. Thiazides are excreted in human breast milk. If use of the combination drug product is deemed essential, the patient should stop nursing.

Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

Adverse Reactions

Side effects observed in association with the use of Triamterene and Hydrochlorothiazide Tablets, other combination products containing triamterene/ hydrochlorothiazide, and products containing triamterene or hydrochlorothiazide include the following:

Gastrointestinal: jaundice (intrahepatic cholestatic jaundice), pancreatitis, nausea, appetite disturbance, taste alter¬ation, vomiting, diarrhea, constipation, anorexia, gastric irritation, cramping.

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Central Nervous System: drowsiness and fatigue, insomnia, headache, dizziness, dry mouth, depression, anxiety, vertigo, restlessness, paresthesias.

Cardiovascular: tachycardia, shortness of breath and chest pain, orthostatic hypotension (may be aggravated by alcohol, barbiturates or narcotics).

Renal: acute renal failure, acute interstitial nephritis, renal stones composed of triamterene in association with other calculus materials, urine discoloration.

Hematologic: leukopenia, agranulocytosis, thrombocytopenia, aplastic anemia, hemolytic anemia and megaloblastosis.

Ophthalmic: xanthopsia, transient blurred vision.

Hypersensitivity: anaphylaxis, photosensitivity, rash, urticaria, purpura, necrotizing angiitis (vasculitis, cutaneous vasculitis), fever, respiratory distress including pneumonitis.

Other: muscle cramps and weakness, decreased sexual performance and sialadenitis.

Whenever adverse reactions are moderate to severe, therapy should be reduced or withdrawn.

Altered Laboratory Findings:

Serum Electrolytes: hyperkalemia, hypokalemia, hyponatremia, hypomagnesemia, hypochloremia (seeWARNINGS,PRECAUTIONS).

Creatinine, Blood Urea Nitrogen: Reversible elevations in BUN and serum creatinine have been observed in hypertensive patients treated with Triamterene and Hydrochlorothiazide tablets.

Glucose: hyperglycemia, glycosuria and diabetes mellitus (seePRECAUTIONS).

Serum Uric Acids, PBI and Calcium: (seePRECAUTIONS).

Other: Elevated liver enzymes have been reported in patients receiving Triamterene and Hydrochlorothiazide tablets.

Overdosage

No specific data are available regarding Triamterene and Hydrochlorothiazide overdosage in humans and no specific antidote is available.

Fluid and electrolyte imbalances are the most important concern. Excessive doses of the triamterene component may be elicit hyperkalemia, dehydration, nausea, vomiting and weakness and possibly hypotension. Overdosing with hydrochlorothiazide has been associated with hypokalemia, hypochloremia, hyponatremia, dehydration, lethargy (may progress to coma) and gastrointestinal irritation. Treatment is symptomatic and supportive. Therapy with Triamterene and Hydrochlorothiazide should be discontinued. Induce emesis or institute gastric lavage. Monitor serum electrolyte levels and fluid balance. Institute supportive measures as required to maintain hydration, electrolyte balance, respiratory, cardiovascular, and renal function.

Triamterene and Hydrochlorothiazide Dosage and Administration

Note: 37.5 mg/25 mg=37.5 mg triamterene and 25 mg hydrochlorothiazide
75 mg/50 mg=75 mg triamterene and 50 mg hydrochlorothiazide

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The usual dosage of Triamterene and Hydrochlorothiazide as a tablet is 37.5 mg/25 mg to 75 mg/50 mg daily, given as a single dose with appropriate monitoring of serum potassium (seeWARNINGS). There is no experience with the use of more than 75 mg/50 mg daily of Triamterene and Hydrochlorothiazide. Clinical experience with the administration of 37.5 mg/25 mg of Triamterene and Hydrochlorothiazide twice daily (in divided doses rather than as a single dose) suggests an increased risk of electrolyte imbalance and renal dysfunction.

Patients receiving 50 mg of hydrochlorothiazide who become hypokalemia may be transferred to this 75 mg/ 50 mg product directly. Patients receiving 25 mg hydrochlorothiazide who become hypokalemic may be transferred to a 37.5 mg/25 mg product directly.

In patients requiring hydrochlorothiazide therapy and in whom hypokalemia cannot be risked, therapy may be initiated with 37.5 mg/25 mg of Triamterene and Hydrochlorothiazide. If an optimal blood pressure response is not obtained with 37.5 mg/25 mg of Triamterene and Hydrochlorothiazide, then the dose should be increased to 75 mg/ 50 mg daily as a single dose. If blood pressure still is not controlled, another antihypertensive agent may be added (seePRECAUTIONS, Drug Interactions).

Clinical studies have shown that patients taking less bioavailable formulations of Triamterene and Hydrochlorothiazide in daily doses of 25 to 50 mg hydrochlorothiazide and 50 to 100 mg of triamterene may be safely changed to 37.5 mg/25 mg of Triamterene and Hydrochlorothiazide daily. All patients changed from less bioavailable formulations to this product should be monitored clinically and for serum potassium after the transfer.

How is Triamterene and Hydrochlorothiazide Supplied

Triamterene and Hydrochlorothiazide Tablets USP, 37.5 mg/25 mg, are light green, round, scored tablets, debossed with Watson 424 and are available in bottles of 30, 100 and 500.

Triamterene and Hydrochlorothiazide Tablets USP, 75 mg/50 mg, are yellow, round, scored tablets, debossed with Watson 348 and are available in bottles of 30, 100, 500 and 1000.

Bottles of 30 and 100 tablets are provided with child-resistant closures.

Store at 20°-25°C (68°-77°F). [See USP controlled room temperature.] Protect from light.

Dispense in a tight, light-resistant container with a child-resistant closure.

Manufactured By:
Watson Pharma Private Limited
Verna, Salcette Goa 403 722 INDIA

Distributed By:
Watson Pharma, Inc.
Corona, CA 92880 USA

Revised: December 2008 1208B
174380

PRINCIPAL DISPLAY PANEL

NDC 0591-0424-01

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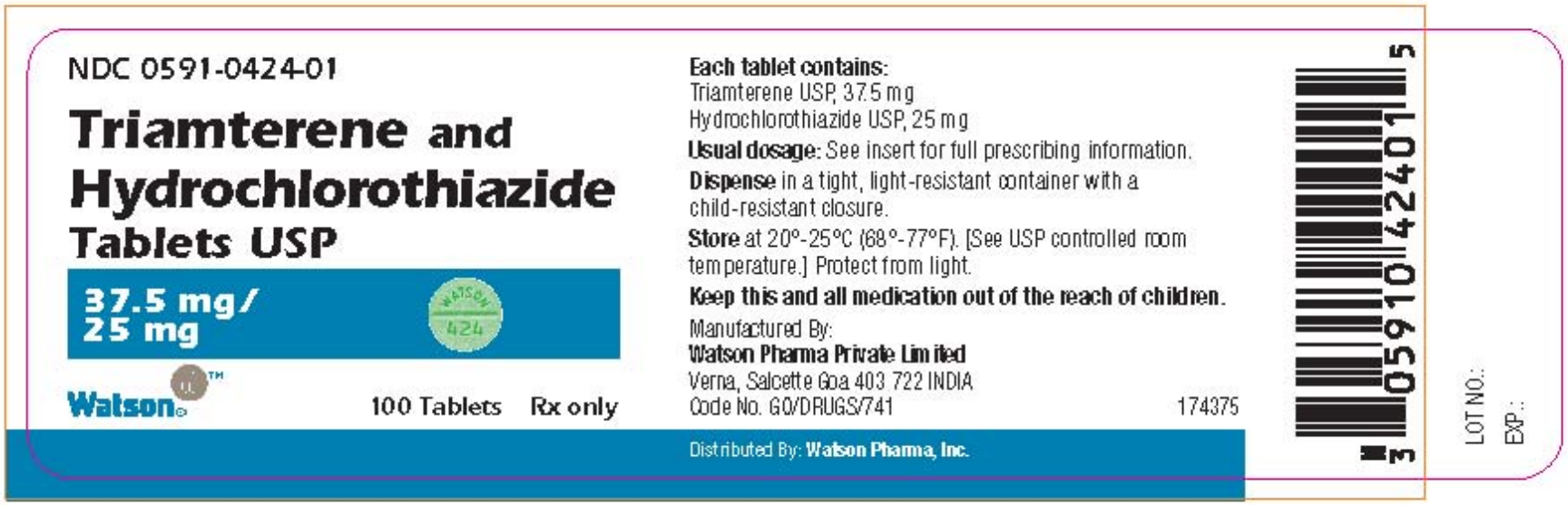
Triamterene and
Hydrochlorothiazide
Tablets USP
37.5mg/25mg
Watson 100 Tablets Rx only

Each tablet contains:
Triamterene USP, 37.5 mg
Hydrochlorothiazide USP, 25 mg
Usual dosage: See insert for full prescribing information.
Dispense in a tight, light-resistant containter with a child-resistant closure.
Store at 20º-25ºC (68º-77ºF). [See USP controlled room temperature.] Project from light.
Keep this and all medication out of the reach of children.

Manufactured By:
Watson Pharma Private Limited
Verna, Salcette Goa 403 722 INDIA
Code No. GO/DRUGS/741 174375
Distributed By: Watson Pharma, Inc



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PRINCIPAL DISPLAY PANEL

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

NDC 0591-0348-01
Triamterene and
Hydrochlorothiazide
Tablets USP
75mg/50mg
Watson 100 Tablets Rx only

Each tablet contains:
Triamterene USP, 75 mg
Hydrochlorothiazide USP, 50 mg
Usual Dosage: See insert for full prescribing information.
Dispense in a tight, light-resistant containter wtih a child-resistant closure.
Store at 20º-25ºC (68º-77ºF). [See USP controlled room temperature.] Project from light.
Keep this and all medication out of the reach of children.

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Code No. GO/DRUGS/741 174377
Distributed By: Watson Pharma, Inc.

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Triamterene and Hydrochlorothiazide

Triamterene and Hydrochlorothiazide tablet

Product Information

Product Type	HUMAN PRESCRIPTION DRUG	NDC Product Code (Source)	0591-0424
Route of Administration	ORAL	DEA Schedule	

Active Ingredient/Active Moiety

Ingredient Name	Basis of Strength	Strength
TRIAMTERENE (TRIAMTERENE)	TRIAMTERENE	37.5 mg
HYDROCHLOROTHIAZIDE (HYDROCHLOROTHIAZIDE)	HYDROCHLOROTHIAZIDE	25 mg

Inactive Ingredients

Ingredient Name	Strength
ANHYDROUS LACTOSE	
CELLULOSE, MICROCRYSTALLINE	
POLACRILIN POTASSIUM	
POLYETHYLENE GLYCOL 8000	
POVIDONE	
MAGNESIUM STEARATE	
FD&C BLUE NO. 2	
ALUMINUM OXIDE	

Product Characteristics

Color	GREEN (Light green)	Score	2 pieces
Shape	ROUND	Size	7mm
Flavor		Imprint Code	WATSON;424
Contains			

Packaging

#	NDC	Package Description	Multilevel Packaging
1	0591-0424-01	100 TABLET In 1 BOTTLE, PLASTIC	None
2	0591-0424-05	500 TABLET In 1 BOTTLE, PLASTIC	None
3	0591-0424-30	30 TABLET In 1 BOTTLE, PLASTIC	None

Marketing Information

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA073449	09/23/1993	

Triamterene and Hydrochlorothiazide
Triamterene and Hydrochlorothiazide tablet

Product Information

Product Type	HUMAN PRESCRIPTION DRUG	NDC Product Code (Source)	0591-0348
Route of Administration	ORAL	DEA Schedule	

Active Ingredient/Active Moiety

Ingredient Name	Basis of Strength	Strength
TRIAMTERENE (TRIAMTERENE)	TRIAMTERENE	75 mg
HYDROCHLOROTHIAZIDE (HYDROCHLOROTHIAZIDE)	HYDROCHLOROTHIAZIDE	50 mg

Inactive Ingredients

Ingredient Name	Strength
ANHYDROUS LACTOSE	
CELLULOSE, MICROCRYSTALLINE	
POLACRILIN POTASSIUM	
POLYETHYLENE GLYCOL 8000	
POVIDONE	
MAGNESIUM STEARATE	

Product Characteristics

Color	YELLOW	Score	2 pieces
Shape	ROUND	Size	10mm
Flavor		Imprint Code	WATSON;348
Contains			

Packaging

#	NDC	Package Description	Multilevel Packaging
1	0591-0348-01	100 TABLET In 1 BOTTLE, PLASTIC	None
2	0591-0348-05	500 TABLET In 1 BOTTLE, PLASTIC	None
3	0591-0348-10	1000 TABLET In 1 BOTTLE, PLASTIC	None
4	0591-0348-30	30 TABLET In 1 BOTTLE, PLASTIC	None

Marketing Information

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA071851	09/23/1993	

Labeler - Watson Laboratories, Inc. (023932721)

Establishment

Name	Address	ID/FEI	Operations
Watson Laboratories, Inc.		840054118	ANALYSIS, LABEL, MANUFACTURE, PACK

Establishment

Name	Address	ID/FEI	Operations
Watson Pharma Private Limited		677605709	ANALYSIS, LABEL, MANUFACTURE, PACK

Revised: 02/2011Watson Laboratories, Inc.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Cisplatin, Continuous-Infusion 5-Fluorouracil, and Intermediate-Dose Methotrexate in the Treatment of Unresectable Non-Small Cell Carcinoma of the Lung

Catherine A. Wheeler, MD, Lawrence N. Shulman, MD,*
Thomas Ervin, MD,† Steven E. Come, MD,
and Lowell E. Schnipper, MD

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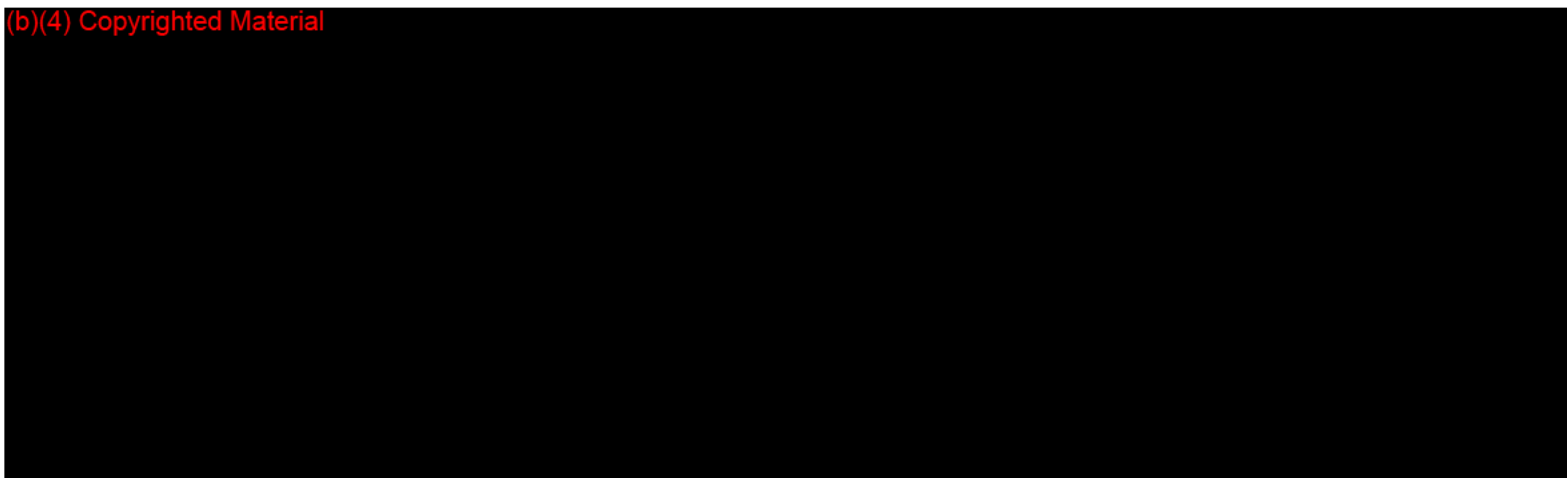

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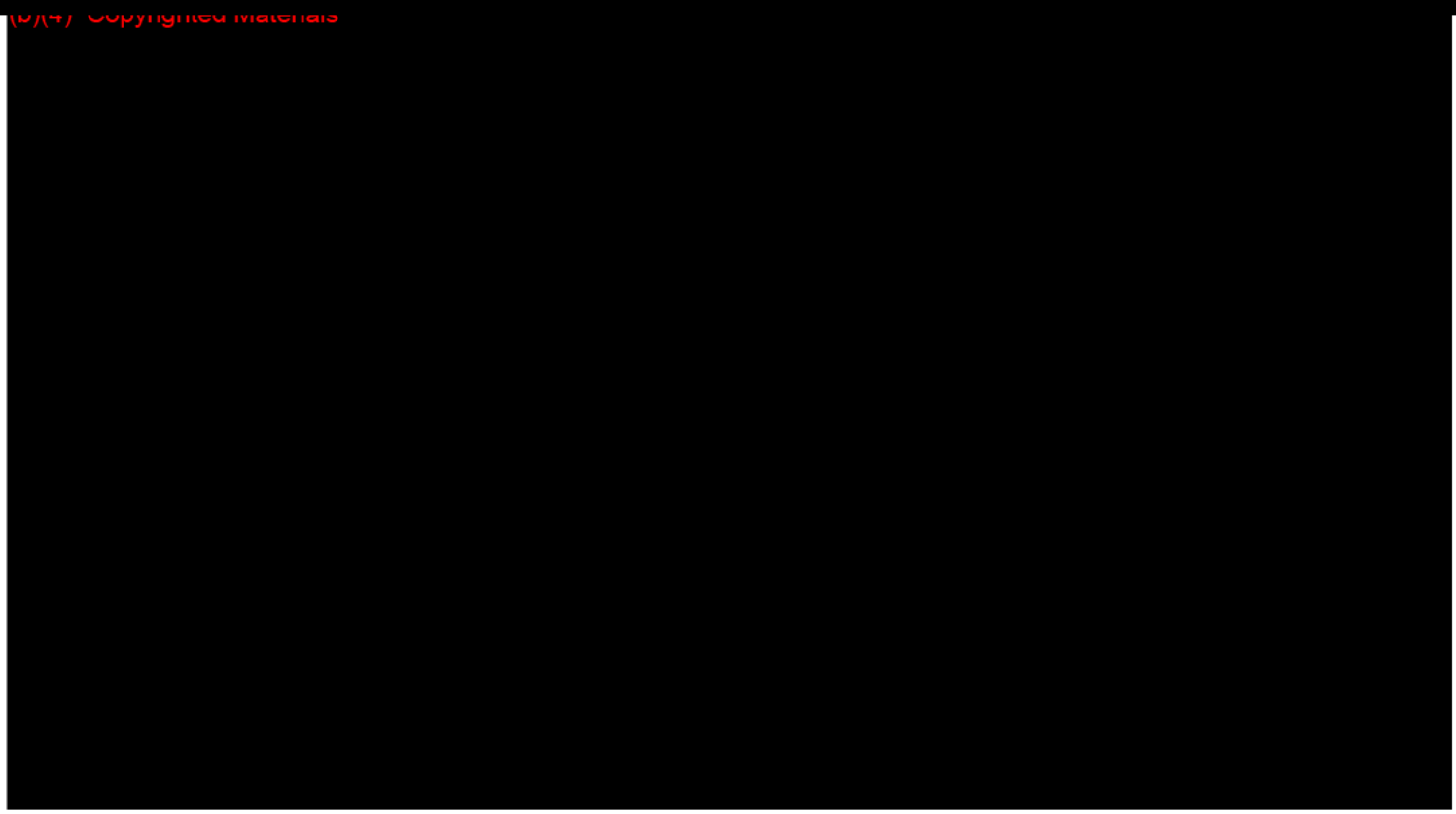

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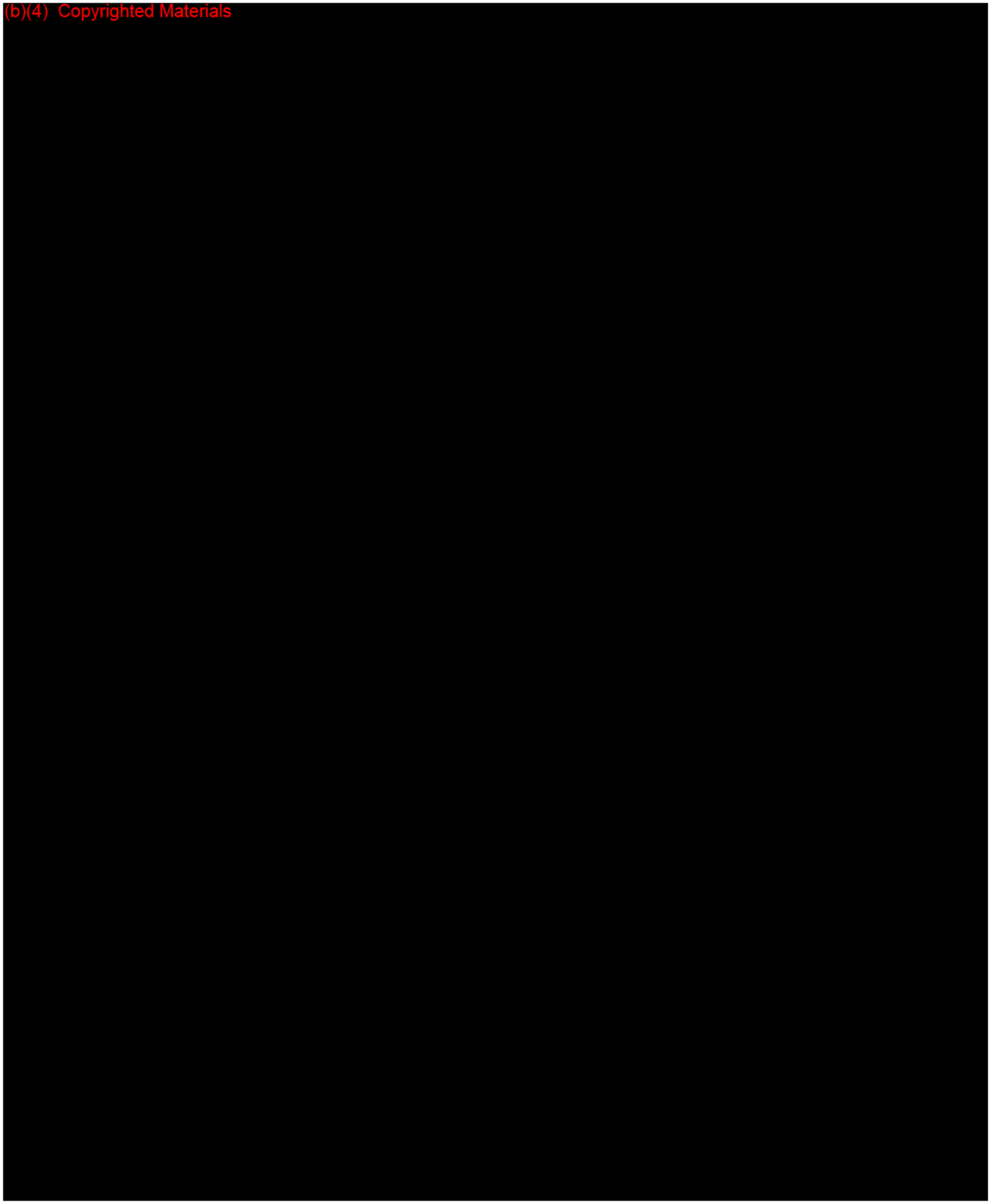

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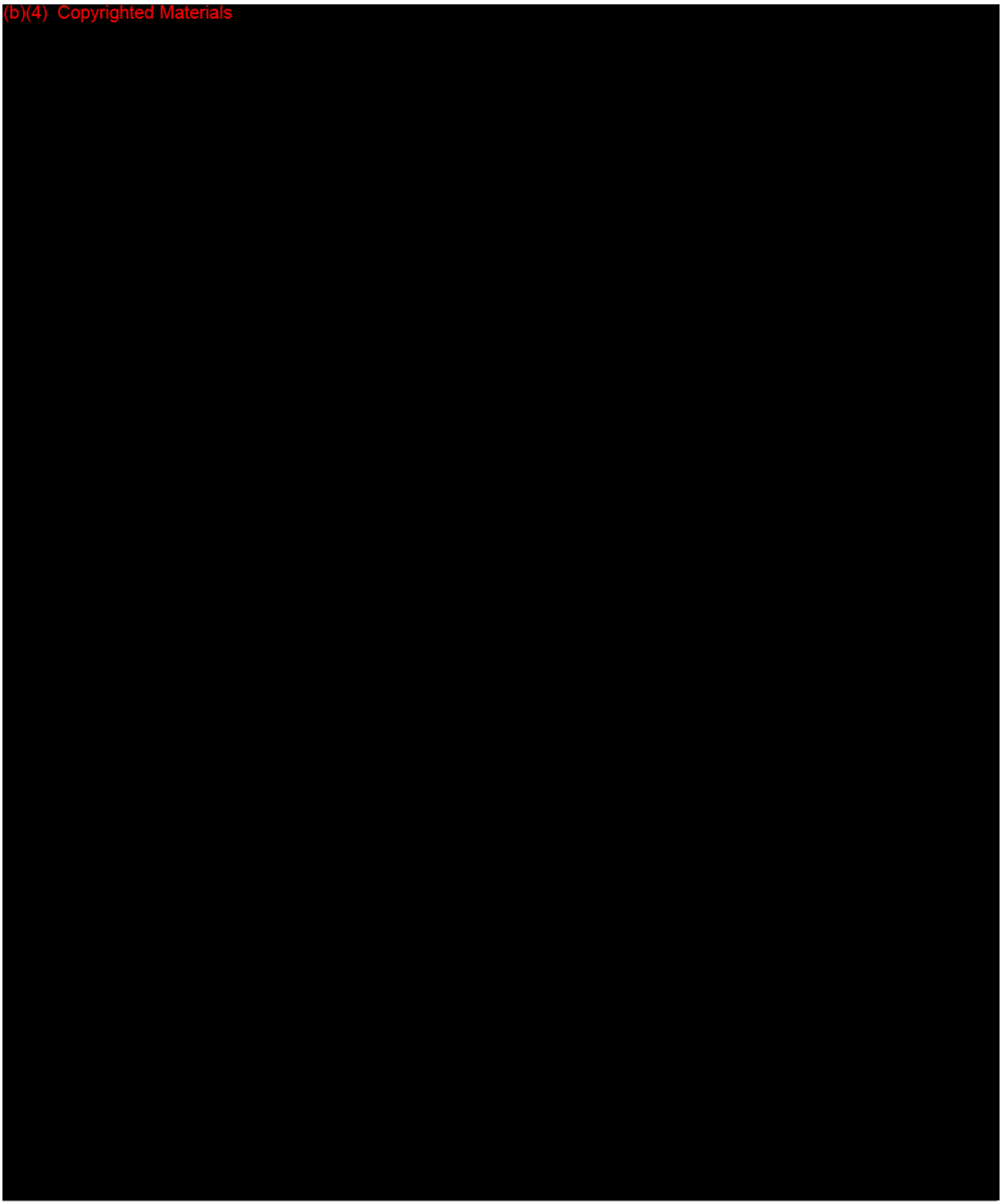

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Carboxypeptidase-G_2 Rescue in a Patient with High Dose Methotrexate-Induced Nephrotoxicity

Brigitte C. Widemann, M.D., Maxine L. Hetherington, M.D., Robert F. Murphy, B.S., Frank M. Balis, M.D., and Peter C. Adamson, M.D.*

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Understanding and Managing Methotrexate Nephrotoxicity

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Key Words. Methotrexate toxicity • Renal dysfunction • Carboxypeptidase-G_2 • Hemodialysis Hemoperfusion • Thymidine • High-dose chemotherapy • Rescue agents

LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Discuss the pharmacology of methotrexate.
2. Describe the current incidence and presentation of high-dose methotrexate-induced renal dysfunction.
3. Discuss conventional and investigational treatment approaches to high-dose methotrexate-induced renal dysfunction.

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ABSTRACT

Methotrexate (MTX) is one of the most widely used anticancer agents, and administration of high-dose methotrexate (HDMTX) followed by leucovorin (LV) rescue is an important component in the treatment of a variety of childhood and adult cancers. HDMTX can be safely administered to patients with normal renal function by the use of alkalinization, hydration, and pharmacokinetically guided LV rescue. Despite these measures, HDMTX-induced renal dysfunction continues to occur in approximately 1.8% of patients with osteosarcoma treated on clinical trials. Prompt recognition and treatment of MTX-induced renal dysfunction are essential to prevent potentially life-threatening MTX-associated toxicities, especially myelosuppression, mucositis, and dermatitis. In addition to conventional treatment approaches, dialysis-based methods have been used to remove MTX with limited effectiveness. More recently carboxypeptidase-G_2 ($CPDG_2$), a recombinant bacterial enzyme that rapidly hydrolyzes MTX to inactive metabolites, has become available for the treatment of HDMTX-induced renal dysfunction. $CPDG_2$ administration has been well tolerated and resulted in consistent and rapid reductions in plasma MTX concentrations by a median of 98.7% (range, 84%–99.5%). The early administration of $CPDG_2$ in addition to LV may be beneficial for patients with MTX-induced renal dysfunction and significantly elevated plasma MTX concentrations. *The Oncologist* 2006;11:694–703

INTRODUCTION

Methotrexate (MTX), a classical antifolate, is one of the most widely used and studied anticancer agents [1–4]. Unlike other anticancer agents, MTX can be safely administered over a wide dose range, ranging from 20 mg/m² per week in maintenance chemotherapy for acute lymphoblastic leukemia and treatment of nononcologic diseases including rheumatoid arthritis or psoriasis [4–6], and when combined with leucovorin (LV) rescue, to doses of 1,000–33,000 mg/m² [7]. The latter, termed high-dose

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Received January 20, 2006; accepted for publication April 21, 2006. ©AlphaMed Press 1083-7159/2006/$20.00/0

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methotrexate (HDMTX) is usually administered as a prolonged i.v. infusion and is an important component in the treatment regimens for a variety of cancers, including acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer [3, 8–11]. HDMTX can be safely administered to patients with normal renal function by vigorously hydrating and alkalinizing the patient to enhance the solubility of MTX in urine and through the use of pharmacokinetically guided LV rescue to prevent potentially lethal MTX toxicity [2, 12].

Despite these preventive measures, MTX-induced nephrotoxicity continues to occur, albeit infrequently. As MTX is primarily cleared by renal excretion, MTX-induced renal dysfunction leads to delayed elimination of MTX, and the resulting sustained, elevated plasma MTX concentration may lead to ineffective rescue by LV and a marked enhancement of MTX's other toxicities [3, 9, 13–15].

Since the introduction of HDMTX with LV rescue more than 25 years ago by Djerassi et al. [16], our ability to safely administer this regimen to patients has improved, and there have been a number of advances in the treatment of HDMTX-induced renal dysfunction over the past 20 years. This report reviews the etiology, incidence, presentation, and treatment of HDMTX-induced renal dysfunction.

Methotrexate Pharmacology

Knowledge of MTX's mechanism of action and metabolism are important for understanding MTX-associated toxicities and treatment. MTX enters the cell via the reduced folate carrier and undergoes polyglutamation catalyzed by folylpolyglutamate synthetase. Once polyglutamated, MTX is retained in cells for prolonged periods of time. Methotrexate and its polyglutamates block de novo nucleotide synthesis primarily by depleting cells of reduced tetrahydrofolate cofactors through inhibition of dihydrofolate reductase (DHFR) (Fig. 1) [17]. MTX polyglutamates and dihydrofolates that accumulate as a result of DHFR inhibition also inhibit thymidylate synthase and other enzymes involved in the purine biosynthetic pathway [18, 19].

Similar to other antimetabolites, critical determinants of MTX cytotoxicity are not only drug concentration but also duration of exposure. High concentrations of MTX may be well tolerated for brief periods of time, whereas prolonged exposure to low concentrations can result in life-threatening toxicity. The type of toxicity observed with MTX is also a function of this concentration–time dependence. Exposure to millimolar concentrations of MTX for minutes to hours may lead to acute renal, central nervous system, and liver toxicity; exposure to MTX concentrations as low as 0.01 and 0.005 μM for >24 hours may result in bone marrow and gastrointestinal epithelial toxicity, respectively [20].

Following administration of HDMTX, two metabolites, 7-hydroxy-methotrexate (7-OH-MTX) and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA), are observed in plasma. Within 12–24 hours of the start of a HDMTX infusion, the plasma concentration of 7-OH-MTX, formed by the action of the enzyme aldehyde oxidase, exceeds the concentration of MTX [21, 22]. Intracellular polyglutamation of 7-OH-MTX results in prolonged retention and enhanced cytotoxicity [23]. DAMPA, a minor, inactive [24–26] metabolite of MTX, accounting for <5% of the total dose of drug that is excreted in urine [24], is presumably formed from MTX that is excreted into the intestinal tract, hydrolyzed by bacterial carboxypeptidases, and then reabsorbed.

Pathogenesis of MTX-Induced Renal Dysfunction

The etiology of MTX-induced renal dysfunction is believed to be mediated by the precipitation of MTX and its metabolites in the renal tubules [21, 22, 27] or via a direct toxic effect of MTX on the renal tubules [17]. More than 90% of MTX is cleared by the kidneys [13]. MTX is poorly soluble at acidic pH, and its metabolites, 7-OH-MTX and DAMPA, are six- to tenfold less soluble than MTX, respectively [21, 24]. An increase in the urine pH from 6.0 to 7.0 results in a five- to eightfold greater solubility of MTX and its metabolites, a finding that underlies the recommendation of i.v. hydration (2.5–3.5 liters of fluid per m^2 per 24 hours, beginning 12 hours before MTX infusion and continuing for 24–48 hours) and urine alkalinization (40–50 mEq sodium bicarbonate per liter of i.v. fluid) prior to, during, and after the administration of HDMTX.

Shorter durations of HDMTX infusions with resultant higher plasma and urinary MTX concentrations may carry an increased risk for renal dysfunction.

Several drugs have been associated with increased toxicity when coadministered with MTX. The most significant interactions involve agents that interfere with MTX excretion, primarily by competing for renal tubular secretion, such as probenecid, salicylates, sulfisoxazole, penicillins, and nonsteroidal anti-inflammatory agents [28–31].

MTX-induced renal dysfunction results in sustained, elevated plasma MTX concentrations, which in turn may lead to ineffective rescue by LV and a marked enhancement of MTX's other toxicities, especially myelosuppression, mucositis, hepatitis, and dermatitis [2, 3, 9, 14, 15].

Vomiting and diarrhea during or shortly after the administration of MTX have been observed in patients who developed MTX toxicity [32, 33], but the majority of patients with renal dysfunction are initially asymptomatic, and most present with nonoliguric renal dysfunction [14, 32, 34]. An abrupt rise in serum creatinine during or shortly

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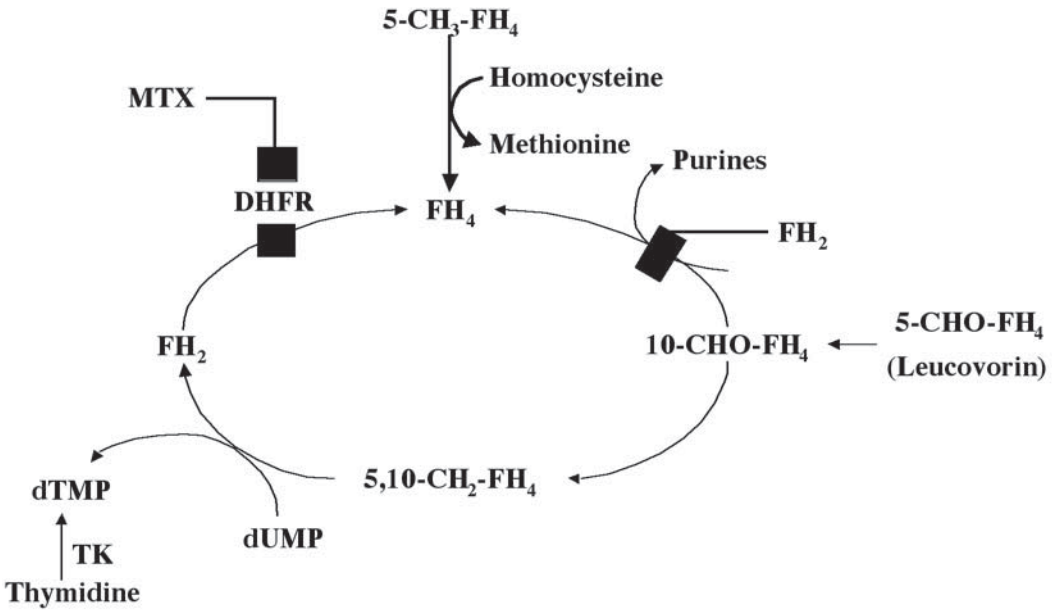

Figure 1. Folate pathway. Sites of action of methotrexate (MTX) and of the rescue agents leucovorin and thymidine. MTX primarily inhibits dihydrofolate reductase (DHFR). This results in the depletion of reduced folates (FH_4), which are required for deoxythymidine monophosphate (dTMP) synthesis from deoxyuridine monophosphate (dUMP), and in accumulation of dihydrofolates (FH_2), which inhibit purine synthesis. The MTX rescue agent leucovorin restores the reduced folate pool after conversion to its active metabolite 5-methyltetrahydrofolate (5-CH_3-FH_4). Thymidine is directly converted to thymidine monophosphate by the enzyme thymidine kinase (TK), thereby circumventing blockade of the de novo pathway by MTX.

after MTX infusion indicates the development of renal dysfunction and can result in significantly elevated plasma MTX concentrations. Although the risk for MTX toxicity is dependent upon the dose and schedule of administration, plasma MTX concentrations should be ≤1.0 μM at 42 hours after the start of the HDMTX infusion, and plasma MTX concentrations ≥10 μM at this time point are associated with a high risk for the development of toxicities [2, 32, 33].

In the absence of early diagnosis based on urine output, serum creatinine, and plasma MTX determination, coupled with intervention that includes pharmacokinetically guided increase in LV rescue, patients present following a delay of several days with severe mucositis, profound bone marrow suppression, and less commonly, dermatitis. Rescue attempts with even very high doses of LV at this symptomatic stage have a small likelihood of relieving MTX toxicities. Significantly elevated liver function tests have been associated with HDMTX administration but do not appear to be associated with the development of renal failure.

Incidence

In the 1970s, prior to routine monitoring of plasma MTX concentrations and pharmacokinetically guided adjustment of LV, the mortality associated with HDTMX infusions ranged between 4.6% and 6% [35–37]. Data from a number of studies performed in the 1970s found that elevated plasma MTX concentrations were predictive for the development of renal toxicities (Table 1). These studies demonstrated that: (a) sustained elevation of plasma MTX concentrations at 24 hours (>5–10 μM), 48 hours (>1.0 μM), and 72 hours (>0.1 μM) after administration of MTX are predictive for the development of toxicity; (b) in the absence of elevated plasma MTX concentrations, the risk for the development of MTX-associated toxicities is minimal; (c) in most circumstances, the development of MTX-associated toxicities can be ameliorated or prevented when patients with elevated plasma MTX concentrations receive pharmacokinetically guided doses of LV rescue. These studies resulted in uniform institution of aggressive hydration, alkalinization, and pharmacokinetically guided LV. Nomograms guiding the duration and degree of rescue with LV based upon plasma MTX concentrations as a function of time of drug administration were developed and are being used in ongoing clinical trials that administer HDMTX (Fig. 2) [38].

Reports of significant morbidity and mortality secondary to HDMTX-induced renal dysfunction, however, continue to appear in the literature [15, 32, 39–41].

With the advent of new therapeutic strategies, we recently reassessed the current incidence of HDMTX-induced renal dysfunction in patients with osteosarcoma, a population that usually is treated with HDMTX administered as a short i.v.

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Table 1. Plasma methotrexate concentrations indicating a high risk for the development of methotrexate (MTX)-associated toxicities

Dosage regimen		No. of patients/ MTX cycles	Defined toxic MTX concentration	No. of toxic concentration/ toxicity	Study
Methotrexate	**Leucovorin**				
1–15 g/m^2 over 20 hrs or 1 g/m^2 as bolus	40 mg/m^2 at hr 24, then 25 mg/m^2 q6h × 12 doses	52/?	> ≈ 0.9 μM at 48 hrs > ≈ 0.5 μM at 72 hrs	?	Tattersall et al. [75]
2 g/m^2 as bolus or over 20 hrs	40 mg/m^2 at hr 24, then 256 mg/m^2 q6h × 12	?/40	>0.1 μM at 72 hrs	7/8	Pitman et al. [76]
50–250 mg/kg over 6 hrs	15 mg/m^2 q6h × 8, start 2 hrs post-MTX (augmented for some pts with MTX >0.9 μM at 48 hrs)	78 /395	>0.9 μM at 48 hrs	12/5	Stoller et al. [12]
8 g/m^2 over 4 hrs	9–15 mg q6h × 12 starting 2 hrs post-MTX	?	>10 μM at 24 hrs >1 μM at 48 hrs >0.1 μM at 72 hrs	74/24 68/25 96/20	Nirenberg et al. [77]
50–300 mg/kg over 4 hrs	40 mg/m^2 4 hrs post-MTX then 15 mg q6h × 11 (augmented for all pts with MTX >10 μM at 24 hrs)	134/496	>10 μM at 24 hrs	83/12	Isacoff et al. [78]
725–15,000 mg/m^2 over 6 hrs	5–90 mg/m^2 q3h × 8 starting 3 hrs post-MTX then 12 mg/m^2 q6h × 8 (augmented for all pts with 24-hr MTX >5 μM)	?/114	>5 μM at 24 hrs and MTX $t_{1/2}$ >3.5 hrs	5/1	Evans et al. [79]

Abbreviations: hr, hour; pts, patients; q3h, every 3 hours; q6h, every 6 hours; $t_{1/2}$, half-life.

infusion separate from cycles that contain other cytotoxic drugs. Our review of the recent literature after 1980, a time during which hydration and alkalinization were administered routinely as part of HDMTX administration, and of clinical trials estimated the incidence of renal dysfunction following HDMTX to be 1.8%. Of 3,887 patients, 68 developed grade ≥2 (World Health Organization criteria) nephrotoxicity. The mortality among patients who developed renal dysfunction was 4.4% [42]. Of note, this estimate only included patients entered in clinical trials, who likely receive optimal supportive care. The incidence of HDMTX-induced renal dysfunction in all patients receiving HDMTX may be higher outside clinical trials and in older patients, as the likelihood of reduced renal function resulting from physiological aging processes and acquisition of comorbidities increases with age. No comparable overview in adult/elderly patients (e.g., patients with primary central nervous system lymphoma) exists, but some available data indicate that the incidence of HDMTX-related nephrotoxicity may be considerably higher in this subgroup of patients [43].

Conventional Treatment Approaches

The cornerstones of preventing HDMTX toxicity—alkalinization, maintaining urine output, monitoring serum creatinine and plasma MTX concentrations, and pharmacokinetically guided LV rescue—are also the cornerstones of management of the patient who develops early signs of renal dysfunction. Renal dysfunction, recognized by a rise in serum creatinine and elevated plasma MTX concentrations, should be initially addressed by promptly increasing the LV dose or schedule based on the time-dependent concentration of MTX (Fig. 2) [2]. Similar to MTX, LV (5-formyltetrahydrofolate) enters the cell via the reduced folate carrier and is converted to its active metabolite 5-methyltetrahydrofolate (5-mTHF) (Fig. 1). The reversal of MTX by LV is competitive, with relatively higher concentrations of LV required as the MTX concentration increases [2, 4]. Hydration and alkalinization should be continued or increased, provided that adequate urine output can be maintained. Other supportive care measures include administering antibiotics, management of fluid and electrolytes, and transfusion of blood products as necessary.

The concern that patients remain at risk for severe MTX toxicity as long as elevated concentrations of MTX persist in the circulation is reflected in the scientific literature, in which methods that attempt to address the underlying problem of impaired MTX elimination have been reported. We reviewed 30 publications published in 1980–2002 on the use and efficacy of dialysis-based methods of MTX removal in 49 patients with HDMTX-induced renal dysfunction [42]. The most frequently used single methods were hemodialysis (n = 10), high-flux hemodialysis (n = 9), and charcoal hemoperfusion or charcoal hemofiltration (n = 7), and 16 patients were treated with multiple modalities. Peritoneal dialysis alone resulted in a minimal decrease in plasma MTX concentrations [44, 45]. The use of other single-modality methods of MTX removal resulted in a median decrease in plasma MTX concentration of 52% (range,

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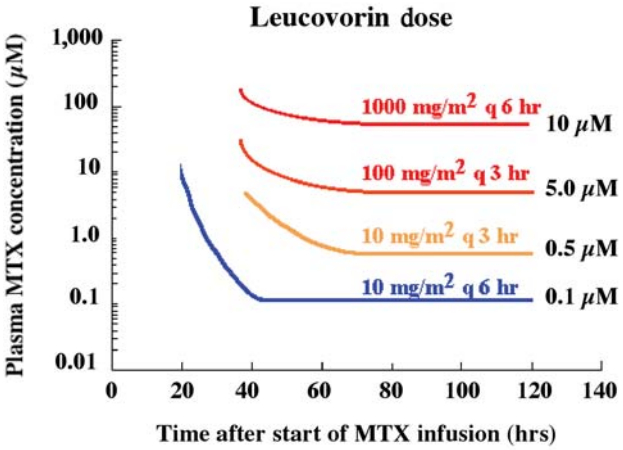

Figure 2. Example of a nomogram for pharmacokinetically guided leucovorin rescue after high-dose methotrexate (MTX) administration. Adapted from Bleyer WA. Therapeutic drug monitoring of methotrexate and other antineoplastic drugs. In: Baer DM, Dita WR, eds. Interpretations in Therapeutic Drug Monitoring. Chicago: American Society of Clinical Pathology, 1981:169–181. ©1981 American Society of Clinical Pathologists.

26%–82%). Dialysis-based methods were used for up to 14 days. The use of high-flux hemodialysis resulted in the greatest decrease in plasma MTX concentrations (median, 75.7%; range, 42%–94%) within the shortest period of time (median, 4 hours; range, 4–12 hours). Only three patients had a >90% decrease in MTX concentration with the use of a single method in one dialysis session [46–48].

A major limitation on the use of dialysis-based methods is the marked rebound in plasma MTX concentrations that can occur when the dialysis is stopped. Rebound increases in the postdialysis plasma MTX concentration were in the range of 10%–221% of the postprocedure MTX level [44, 49, 50] and 90%–100% of the preprocedure MTX level [51–54]. Further limitations of these methods are the accompanying risks for complications from these invasive procedures. Reported complications include cardiac arrest in one patient after plasma exchange [51], bleeding from the catheter exit site [52], anemia [52, 55], thrombocytopenia [52, 56], and hypokalemia and severe hypophosphatemia [48].

Investigational Treatment Approaches

Thymidine (Thd) is an endogenous nucleoside that can effectively circumvent MTX toxicity in patients with normal renal function [57]. Unlike LV, Thd does not compete with MTX for transport into the cell but is directly converted to thymidine monophosphate by the salvage enzyme thymidine kinase, thereby circumventing blockade of the de novo pathway by MTX (Fig. 3) [58]. Repletion of deoxythymidine monophosphate (dTMP) and consequently of thymidine triphosphate pools allows restoration of DNA synthesis.

In humans, the half-life of Thd is approximately 10 minutes, and thus this investigational drug needs to be administered as a continuous i.v. infusion in order to maintain effective plasma concentrations [59]. Thd has been used in 16 patients with MTX-induced renal dysfunction as a rescue agent in combination with LV [14, 58]. Severe toxicity was observed in only three patients, in whom Thd was initiated 5, 12, and 13 days after the start of MTX infusion. The development of Thd as an investigational agent by the National Cancer Institute (NCI) Cancer Therapy Evaluation Program (CTEP) was recently discontinued, and thus Thd is currently not available for investigational use.

The carboxypeptidase-G class of enzymes hydrolyze the terminal glutamate from naturally occurring folates and folate analogs, such as MTX [60]. Carboxypeptidase-G rapidly converts MTX to the inactive metabolites DAMPA and glutamic acid, thus providing an alternate route of elimination to renal excretion. In the 1970s carboxypeptidase-G_1, extracted from *Pseudomonas stutzeri* [61, 62], effectively lowered plasma MTX concentrations in a small number of patients with brain tumors who had been treated with HDMTX [63, 64]. The bacterial source of $CPDG_1$, however, was lost, and no additional patients were treated [65].

Subsequently, carboxypeptidase-G_2 ($CPDG_2$, glucarpidase), a recombinant form of the bacterial enzyme $CPDG_2$, cloned from *Pseudomonas* strain RS-16, has become available and is being developed by CTEP and by Protherics Inc. (Brentwood, TN). It hydrolyzes the glutamate residue from naturally occurring and synthetic folate analogues [66, 67]. When administered to patients with HDMTX-induced renal dysfunction, $CPDG_2$ metabolizes circulating MTX to the inactive metabolite DAMPA (Fig. 3), thus providing an alternate route of elimination to renal excretion. In 21 patients with MTX-induced renal dysfunction treated on a compassionate-use protocol of the NCI, $CPDG_2$ lowered plasma MTX concentrations within 15 minutes of administration by >98% [34, 68]. This group of patients received Thd in addition to $CPDG_2$, because of the concern that $CPDG_2$ could hydrolyze both LV and its active metabolite, 5-mTHF. $CPDG_2$ and Thd rescue were well tolerated in these patients, and MTX-related toxicities were mild to moderate.

To assess the role of Thd in these patients, this study was subsequently amended to restrict Thd administration to patients with prolonged (>96 hours) exposure to MTX or with severe toxicity at study entry. MTX-associated toxicities and outcome were compared in 44 patients who did and 56 patients who did not receive Thd. That study demonstrated that $CPDG_2$ and LV rescue without Thd effectively rescued patients with HDMTX-induced renal dysfunction, provided $CPDG_2$ was administered within 96 hours of the start of the MTX infusion [69].

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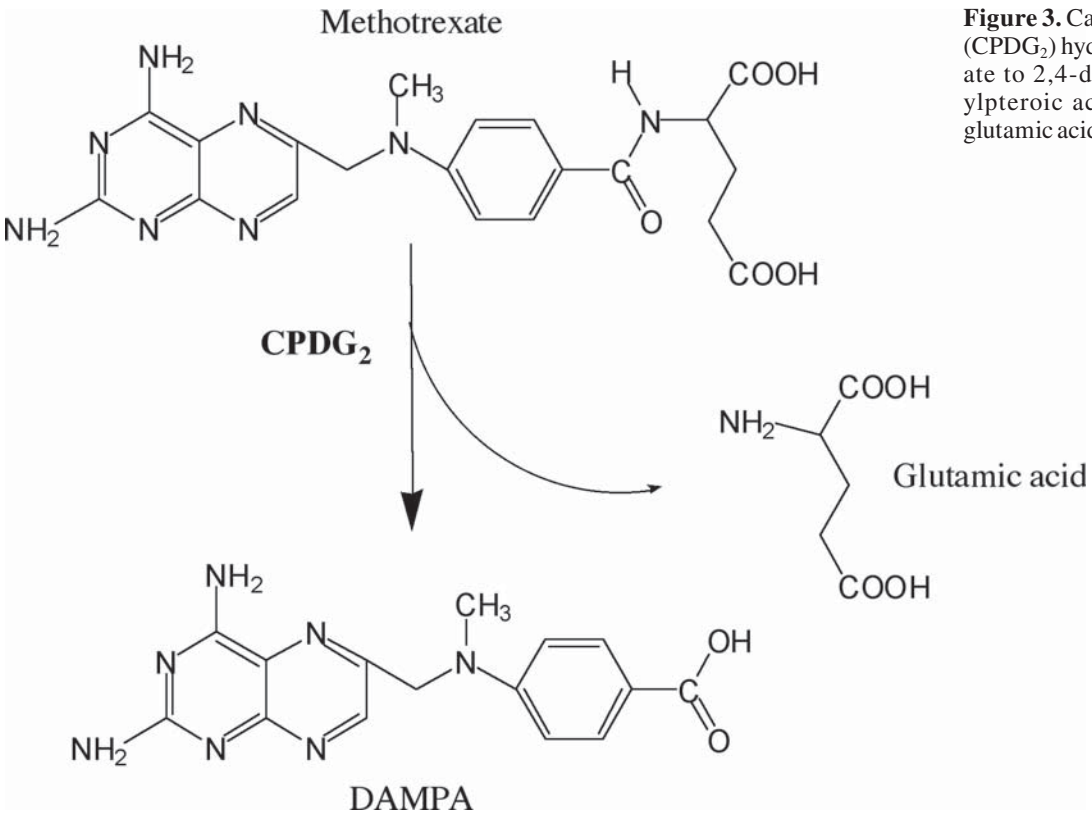

Figure 3. Carboxypeptidase-G_2 ($CPDG_2$) hydrolyzes methotrexate to 2,4-diamino-N^{10}-methylpteroic acid (DAMPA) and glutamic acid.

The efficacy in rapidly lowering plasma MTX concentrations was confirmed in a European study [70], in which LV and $CPDG_2$ were administered to 82 patients with HDMTX-induced renal dysfunction. $CPDG_2$ was administered at a median of 52 hours (range, 25–178 hours) following the start of the MTX infusion and resulted in a 97% (range, 73%–99%) reduction in plasma MTX concentrations.

While $CPDG_2$ preferentially hydrolyzes MTX (K_m of 8 μM), it can also hydrolyze LV (K_m of 120 μM) and its active metabolite 5-mTHF (K_m of 35 μM). The effect of $CPDG_2$ on MTX, LV, and 5-mTHF plasma concentrations was assessed in 11 patients using reverse-phase high-performance liquid chromatography [71]. Although LV concentrations were maintained following $CPDG_2$ administration, LV was likely in the form of the inactive d-isomer. The active metabolite of LV, 5-mTHF, was indeed a substrate for $CPDG_2$ and was effectively hydrolyzed. These findings formed the basis for the recommendation to continue with the administration of high doses of LV (250 mg/m^2 every 6 hours) for 48 hours after $CPDG_2$ to allow for restoration of the intracellular reduced folate pool. Hempel et al. [72] recently evaluated the effect of $CPDG_2$ on the inactive d-isomer and the active l-isomer of LV in vitro, and demonstrated that the active l-isomer was degraded much faster than the d-isomer.

After systemic $CPDG_2$ administration, DAMPA plasma concentrations are similar to pre-$CPDG_2$ MTX concentrations. Persistently high concentrations of the poorly water soluble DAMPA could theoretically lead to further renal toxicity by precipitation in the renal tubules. To evaluate whether DAMPA could contribute to a delay in renal recovery, we studied 20 patients who received $CPDG_2$ and Thd for MTX-induced renal dysfunction. In this population, serum creatinine returned to normal values at a median of 22 days after administration of MTX [34]. This time period is similar to the time to renal recovery seen in patients with HDMTX-induced renal dysfunction and a comparable degree of renal injury who were treated with conventional dialysis-based methods [42]. $CPDG_2$ administration, therefore, does not appear to impact negatively upon the recovery of renal function.

Interestingly, following administration of $CPDG_2$ for MTX-induced renal dysfunction, plasma DAMPA concentrations decline more rapidly than MTX concentrations, suggesting a nonrenal elimination of DAMPA [34]. In a nonhuman primate study of DAMPA metabolism, DAMPA was found to be metabolized to hydroxy-DAMPA, DAMPA-glucuronide, and hydroxy-DAMPA-glucuronide [25]. These metabolites were also identified in patients who received $CPDG_2$ for MTX-induced renal toxicity.

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Metabolism of DAMPA thus likely underlies the more rapid elimination of DAMPA relative to MTX in patients with MTX-induced renal dysfunction treated with $CPDG_2$ [25].

Current Controversies

Is Current Supportive Treatment of HDMTX-Induced Renal Failure Adequate?

Although the use of alkalinization and hydration with HDMTX greatly diminishes the risk for significant nephrotoxicity, we have estimated that approximately 1.8% of patients treated with HDMTX on clinical studies with optimal supportive care still develop renal dysfunction that may prove life threatening. With supportive treatment only, patients remain at risk for severe toxicity as long as elevated concentrations of MTX persist in the circulation. High concentrations of LV may be inadequate in this situation. This clinical observation is supported by laboratory studies that demonstrated that reversal of MTX by LV is competitive, with relatively higher concentrations of LV required as the MTX concentration increases. When concentrations of MTX reached 100 μM, even tenfold higher LV concentrations (1,000 μM) were unable to protect bone marrow cells from toxicity [73].

In a recent single-institution retrospective review, administration of high doses of LV within 24–48 hours of HDMTX administration rescued 13 patients with HDMTX-induced renal dysfunction [74]. A significant proportion of the 13 patients developed neutropenia ($n = 8$) (absolute neutrophil count [ANC] <1,000/μl), thrombocytopenia ($n = 7$) (platelet count <100,000/μl), and mucositis ($n = 6$). The authors concluded that treatment with high doses of LV administered within 24–48 hours after the start of the MTX infusion is sufficient therapy in patients with MTX-induced renal dysfunction. Based on a lower median MTX plasma concentration at 48 hours (16.3 μM) and a lower median peak serum creatinine concentration (2.0 mg/dl), compared with 20 patients who received $CPDG_2$ as a rescue agent (median MTX concentration at 46 hours, 201 μM; median peak serum creatinine, 3.7 mg/dl) [34], the patients reported by Flombaum and Meyers [74] may have suffered from less severe MTX-associated nephrotoxicity. Close monitoring and the ability to intervene early at this single institution likely contributed to the relative success of this approach, although it must be recognized that, despite this early intervention, significant toxicities occurred.

The development of renal dysfunction following MTX administration remains a significant management challenge. The multitude of dialysis-based methods directed at lowering plasma MTX concentrations following development of nephrotoxicity attests to the difficulty encountered by clinicians caring for such patients. The dialysis based methods are relatively inefficient at reducing plasma MTX concentrations, and sole reliance on administering elevated doses of LV is not sufficient treatment for many patients. More effective management strategies could result in better outcomes for these patients.

Risk Versus Benefits of $CPDG_2$

Current treatment for patients who develop HDMTX-induced renal dysfunction relies on high doses of LV to reduce the risk for toxicity, and with markedly elevated plasma MTX concentrations, the use of a dialysis-based methods of drug removal is often attempted. These charcoal hemodialysis/filtration-based methods must be continuously applied, or repeated on a daily basis in order to reduce plasma MTX concentrations to a range in which standard doses of oral LV can be administered. The risks associated with charcoal hemodialysis/filtration-based methods include the risks associated with the insertion of vascular access devices, the risk for bleeding secondary to heparinization and thrombocytopenia, and the risks associated with multiple transfusions of blood products.

The benefits of $CPDG_2$ administration include a >98% decrease in plasma MTX concentration within minutes of enzyme administration. Patients can then be safely managed with LV rescue alone. Early administration of $CPDG_2$ may diminish the risk for serious to life-threatening MTX toxicity. Furthermore, patients avoid the risks associated with charcoal hemodialysis/filtration-based methods of MTX removal, which may not be readily available outside of major medical centers.

The risks of $CPDG_2$ administration appear to be infrequent and minor in nature. Four of 21 patients treated with $CPDG_2$ described readily reversible side effects consisting of a feeling of warmth ($n = 2$), tingling in the fingers ($n = 1$), flushing ($n = 2$), shaking ($n = 1$), and head pressure ($n = 1$) [34], and only 2 of 82 patients in a European study of $CPDG_2$ for HDMTX-induced renal dysfunction described mild and completely reversible symptoms of flushing ($n = 2$) and shaking ($n = 1$) [70]. The theoretical risk for a worsening of renal function secondary to accumulation of the poorly water soluble MTX metabolite DAMPA has not been borne out, as the time to renal recovery is no different than that of historical controls treated with charcoal hemodialysis/filtration-based methods [42]. Even though $CPDG_2$ hydrolyzes MTX preferentially, 5-mTHF, the active metabolite of LV, is also hydrolyzed. Continuing to administer higher doses of LV for 48 hours after administration of $CPDG_2$ is therefore currently recommended and appears effective.

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Recommendations

The lack of early clinical symptoms predicting the development of renal dysfunction emphasizes the need for routine daily monitoring of plasma MTX concentrations and serum creatinine after the administration of HDMTX, until MTX has declined to levels allowing discontinuation of LV (<0.05 to 0.1 μM). After the diagnosis of renal dysfunction has been established, a prompt increase in LV based on plasma MTX concentrations is critical for successful management.

Patients who develop renal dysfunction and have plasma MTX concentrations ≤10 μM at 42–48 hours after the start of the MTX infusion can likely be successfully managed with LV administration and supportive care. Although very early institution of high-dose LV alone may be sufficient for patients with MTX concentrations >10 μM at 42–48 hours, $CPDG_2$ offers a rapid means of decreasing plasma MTX concentrations by 1–2 logarithms within minutes of administration. The risks of $CPDG_2$ toxicity appear minimal, but as with any foreign protein, anaphylactic reactions could theoretically occur. Continuation of high-dose LV for 48 hours following $CPDG_2$ administration is advised to replete intracellular reduced folate pools. After that time, LV rescue should be modified based on the lowered plasma MTX concentrations. The most commonly used commercially available fluorescence polarization immunoassay and all immunoassays significantly overestimate plasma MTX concentrations after administration of $CPDG_2$, because of the crossreactivity of DAMPA in these assays, and results obtained with these assays will not fully reflect the ability of $CPDG_2$ to hydrolyze MTX [26]. As DAMPA is cleared more rapidly than MTX in patients with renal dysfunction, the accuracy of immunoassay results increases within a few days of $CPDG_2$ administration.

For patients with HDMTX-induced renal dysfunction with sustained MTX concentrations >10 μM at 42–48 hours after the start of the MTX infusion, we would recommend that $CPDG_2$ be administered in addition to LV. For patients with renal dysfunction and plasma MTX concentrations of 1–10 μM, administration of $CPDG_2$ may be considered, but fewer data are available for this population. $CPDG_2$ is an investigational drug that can be obtained on a compassionate use protocol from CTEP at the following address: Pharmaceutical Management Branch, CTEP, NCI, Executive Plaza North, Room 7147, 9000 Rockville Pike, Bethesda, Maryland 20892-7422, USA. Telephone: 301-496-5725.

Acknowledgments

We thank Dr. Dale Shoemaker, Matthew Boron, Dr. Aiman Shalabi, and Dr. Percy Ivy at the Cancer Therapy Evaluation Program, National Cancer Institute, for their assistance in making $CPDG_2$ and Thd available for investigational use, and Dr. Roger Melton and the late Dr. Roger Sherwood of Duramed, Ltd., Oxford, UK, for their research efforts and commitment to the development of $CPDG_2$.

This research was supported (in part) by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Disclosures of Potential Conflicts of Interest

Peter Adamson has acted as a consultant for and received support from Protherics, the manufacturer of $CPDG_2$.

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Understanding and Managing Methotrexate Nephrotoxicity

Brigitte C. Widemann and Peter C. Adamson

Oncologist 2006;11;694-703
DOI: 10.1634/theoncologist.11-6-694

This information is current as of August 26, 2010

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VOLUME 28 · NUMBER 25 · SEPTEMBER 1 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Glucarpidase, Leucovorin, and Thymidine for High-Dose Methotrexate-Induced Renal Dysfunction: Clinical and Pharmacologic Factors Affecting Outcome

Brigitte C. Widemann, Frank M. Balis, AeRang Kim, Matthew Boron, Nalini Jayaprakash, Aiman Shalabi, Michelle O'Brien, Michelle Eby, Diane E. Cole, Robert F. Murphy, Elizabeth Fox, Percy Ivy, and Peter C. Adamson

From the National Cancer Institute, Bethesda, MD; and the Children's Hospital of Philadelphia, Philadelphia, PA.

Submitted August 28, 2009; accepted May 27, 2010; published online ahead of print at www.jco.org on August 2, 2010.

Supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research, Bethesda, MD.

Presented in part at the 41st Annual Meeting of the American Society of Clinical Oncology, May 13-17, 2005, Orlando, FL.

The views expressed do not necessarily represent views of the National Institutes of Health or the US government.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/10/2825-3979/$20.00

DOI: 10.1200/JCO.2009.25.4540

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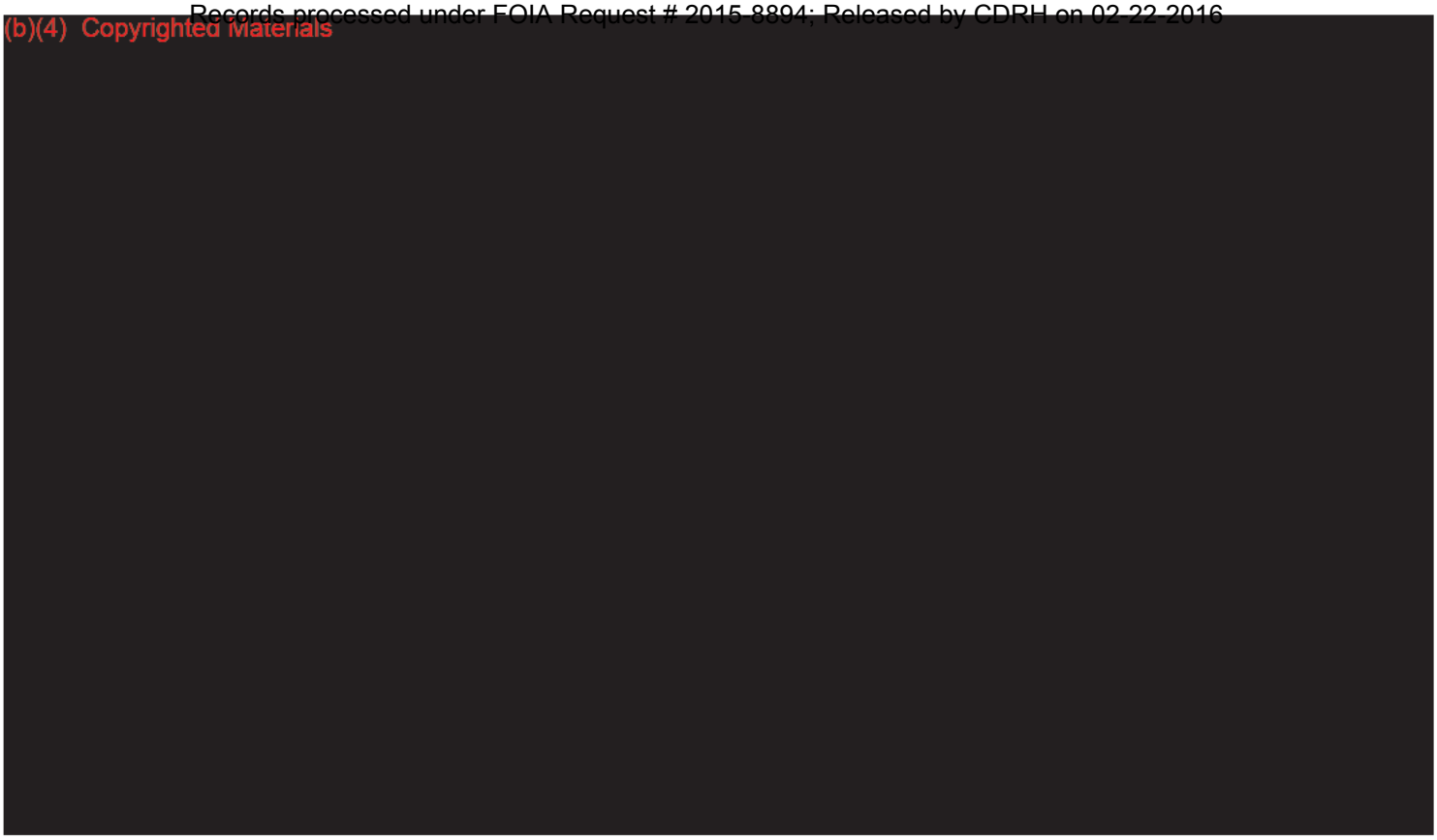

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Eur J Clin Pharmacol (1990) 39: 377–383

European Journal of Clinical Pharmacology

© Springer-Verlag 1990

Pharmacokinetic study of methotrexate, folinic acid and their serum metabolites in children treated with high-dose methotrexate and leucovorin rescue

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Received: August 3, 1989/Accepted in revised form: March 20, 1990

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Food and Drug Administration
Office of Device Evaluation &
Office of In Vitro Diagnostics

COVER SHEET MEMORANDUM

From: Reviewer Name Kellie B. Kelm

Subject: 510(k) Number k111904/S001

To: The Record

Please list CTS decision code CS

☐ Refused to accept (Note: this is considered the first review cycle, See Screening Checklist http://eroom.fda.gov/eRoomReg/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)
☐ Hold (Additional Information or Telephone Hold).
☒ Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

☐	NO	NSE for lack of predicate
☐	NI	NSE for new intended use
☐	NQ	NSE for new technology that raises new questions of safety and effectiveness
☐	NP	NSE for lack of performance data
☐	NC	NSE call for PMAs
☐	NS	NSE no response
☐	NH	NSE for another reason

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	*Attach IFU*	X	
510(k) Summary /510(k) Statement	*Attach Summary*	X	
Truthful and Accurate Statement.	*Must be present for a Final Decision*	X	
Is the device Class III?			X
If yes, does firm include Class III Summary?	*Must be present for a Final Decision*		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)		X	
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReg/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC			X
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			X
Is this device intended for pediatric use only?			X
Is this a prescription device? (If both prescription & OTC, check both boxes.)		X	
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?		X	
Is clinical data necessary to support the review of this 510(k)? Did the application include a completed FORM FDA 3674, *Certification with Requirements of ClinicalTrials.gov Data Bank*? (If not, then applicant must be contacted to obtain completed form.)		X	
Does this device include an Animal Tissue Source?			X
All Pediatric Patients age<=21			X

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Neonate/Newborn (Birth to 28 days)		X
Infant (29 days -< 2 years old)		X
Child (2 years -< 12 years old)		X
Adolescent (12 years -< 18 years old)		X
Transitional Adolescent A (18 - <21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)		X
Transitional Adolescent B (18 -<= 21; No special considerations compared to adults => 21 years old)		X
Nanotechnology		X
Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, http://www.fda.gov/cdrh/comp/guidance/169.html) *Contact OC.*		X

Regulation Number	**Class***	**Product Code**
N/A	Unclassified	LAO

(*If unclassified, see 510(k) Staff)

Additional Product Codes: DLJ, LAS.

Review: [signature] DCTD 10-17-11
(Branch Chief) (Branch Code) (Date)

Final Review: [signature] 10/18/11
(Division Director) (Date)

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510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k111904

B. Purpose for Submission:

New assay, calibrator and control

C. Measurand:

Methotrexate

D. Type of Test:

Quantitative Homogeneous Enzyme Immunoassay

E. Applicant:

ARK Diagnostics, Inc.

F. Proprietary and Established Names:

ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, ARK™ Methotrexate Control

G. Regulatory Information:

1. Regulation section:

Product Code	Classification	Regulation Section	Panel
LAO - Methotrexate enzyme immunoassay	II	Unclassified	Toxicology (91)
DLJ - Clinical toxicology calibrator	II	21 CFR §862.3200	Toxicology (91)
LAS - Clinical toxicology control material	I, reserved	21 CFR §862.3280	Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in

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monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK Methotrexate Calibrator is intended for the calibration of the ARK Methotrexate Assay.

The ARK Methotrexate Control is intended for the quality control of the ARK Methotrexate Assay.

3. Special conditions for use statement(s):

   For prescription use only.

   Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should not be tested with the ARK Methotrexate Assay.

4. Special instrument requirements:

   Performance characteristics were established on the Roche/Hitachi 917 automated clinical chemistry analyzer.

I. Device Description:

The ARK Methotrexate Assay consists of reagents R1 (anti-Methotrexate rabbit polyclonal antibody with substrate) and R2 (Methotrexate labeled with bacterial G6PDH enzyme.)

The ARK Methotrexate Calibrator consists of a six-level set to calibrate the assay. The calibrators consist of a synthetic protein matrix and the levels are 0.00 umol/L, 0.05 umol/L, 0.15 umol/L, 0.25 umol/L, 0.50 umol/L and 1.2 umol/L.

The ARK Methotrexate Control consists of a six-level set used for quality control of the assay (tri-level calibration range set and tri-level high range set). The controls consist of a synthetic protein matrix and the levels are 0.07 umol/L, 0.40 umol/L, 0.80umol/L, 5 umol/L, 50 umol/L and 500 umol/L.

The ARK Methotrexate Dilution Buffer is equivalent to zero calibrator (Calibrator A).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Abbott TDx®/TDxFLx® METHOTREXATE II

2. Predicate K number(s):

   k932615

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3. Comparison with predicate:

Similarities		
Item	Proposed Device	Predicate Device (k932615)
Intended Use/Indications for use	For the quantitative determination of methotrexate. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.	Same
Specimen type	Serum and plasma	Same
Number of calibrators and controls	6 levels (each)	Same
Reagent matrix and storage condition	Liquid, 2-8° C	Same

Differences		
Item	Device	Predicate
Methodology	Homogenous enzyme immunoassay (EIA)	Fluorescence polarization immunoassay (FPIA)
Reagent Components	Two (2) reagent system: Anti-Methotrexate Antibody/Substrate Reagent (R1) (contains rabbit polyclonal antibodies to Methotrexate) Enzyme Reagent (R2) (contains Methotrexate labeled with bacterial G6PDH)	Reagent Pack: W (Wash solution) S (containing Methotrexate antibody (mouse monoclonal)) T (Methotrexate Fluorescein Tracer) P (Pretreatment Solution)
Matrix for calibrators and controls	Synthetic matrix	Human serum

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K. Standard/Guidance Document Referenced (if applicable):

- CLSI document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline*
- CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Guideline EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples*
- CLSI Protocol EP7-A2: *Interference Testing in Clinical Chemistry*
- CLSI Protocol EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*

L. Test Principle:

The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and Methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

M. Performance Characteristics (if/when applicable):

Performance was validated on the Hitachi 917 instrument.

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision was determined as described in CLSI Guideline EP5-A2. Data for the precision evaluation studies (total and within-laboratory precision) were collected on a single Roche/Hitachi 917 automated clinical chemistry analyzer. Each level of control and patient sample was assayed in quadruplicate twice a day over twenty non-consecutive days. A total of 160 determinations were made for each sample. Four calibrations were performed during this interval. The ARK Methotrexate Controls (three levels) and three patient sample pools were tested in each run.

   Mean methotrexate concentration, standard deviation (SD) and coefficients of variation (% CVs) were calculated for within-run, between-day, and total precision.

   Patient specimens that contained methotrexate were pooled to create three levels with sufficient volume to complete the 20-day protocol.

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Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
Controls								
Low	160	0.06	0.005	8.2	0.005	7.3	0.007	10.7
Mid	160	0.37	0.011	3.0	0.008	2.1	0.014	3.8
High	160	0.76	0.032	4.3	0.030	4.0	0.045	5.9
Patient Pools								
Low	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
Mid	160	0.41	0.013	3.3	0.026	6.4	0.029	7.2
High	160	0.82	0.037	4.5	0.043	5.2	0.057	7.0

*Samples were diluted in ARK Methotrexate Dilution Buffer. Mean result and SD were multiplied by the dilution factor.

The sponsor also performed a precision study to evaluate serum and plasma samples. The study was modified from the recommendations in CLSI Guideline EP5-A2; 5 days of precision studies were performed. The results showed that precision for serum and plasma was not significantly different.

b. *Linearity/assay reportable range:*

Linearity studies were performed as suggested in CLSI Guideline EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach.*

Linearity studies were performed by testing concentrations of methotrexate across the claimed measuring range. Gravimetric addition of pure methotrexate (USP) to dimethylformamide and volumetric addition of this stock solution to human serum negative for methotrexate was made to achieve a concentration (HIGH Linearity Sample; 1.30 µmol/L) above the calibration range as suggested in CLSI Protocol EP-6A.

Dilutions of this 1.30 µmol/L HIGH Linearity Sample were made proportionally with pooled human serum negative for methotrexate. Two separately calibrated analytical runs of three replicates of each sample per run were assayed (N=6).

The results of the six replicates were averaged. Regression analyses were performed between the measured mean methotrexate and the theoretical values for each dilution, using first order and second order polynomial determinations. Predicted 1st and 2nd order regressed values were calculated from the regression equations and compared.

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Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30	1.19	1.228	1.199	-2.3 %

Linear regression analysis yields the following result:
$y = 0.9407x + 0.086$
$R^2 = 0.9978$

There are no concentrations at which the percent difference was more than 10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L.

The claimed measuring range of this device is 0.04 - 1.20 µmol/L.

Evaluation of recovery:

An accuracy-by-recovery study was conducted to determine the trueness of quantitative measurements of methotrexate across the calibration range of the ARK™ Methotrexate Assay. Recovery of methotrexate was studied with two sources of methotrexate for comparison.

Samples were prepared by volumetric addition of methotrexate (Cerilliant Certified Stock solution 99.8% purity) to human serum negative for methotrexate. Drug concentrations across the assay range (0.06, 0.10, 0.30, 0.60, and 1.00 µmol/L) were tested. Each sample was assayed in triplicate in each of two separately calibrated runs for a total of six replicates. The results were averaged and compared to the theoretical target concentration and percentage recovery calculated.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Target concentration}}$$

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For comparison, samples were prepared by gravimetric addition of methotrexate (USP > 99.9% purity) to dimethylformamide (DMF) and volumetric addition of this stock solution to human serum negative for methotrexate, then assayed as described above.

Recovery was acceptable if the mean methotrexate concentration measured was within ±10% of the target level for concentrations at >0.1 µmol/L and ±0.01 at ≤0.1 µmol/L.

Target Concentration (µmol/L)	Cerilliant Methotrexate		USP Methotrexate	
	Mean Recovered Concentration (µmol/L)	Recovery (%)	Mean Recovered Concentration (µmol/L)	Recovery (%)
0.06	0.067	+0.007 µmol/L (111.1%)	0.060	100.0
0.10	0.100	100.0	0.098	98.3
0.30	0.295	98.3	0.282	93.9
0.60	0.613	102.2	0.597	99.4
1.00	0.988	98.8	1.008	100.8

Evaluation of samples above the claimed measuring range:
Frequently, patient samples may have methotrexate levels higher than the claimed measuring range of the device. They require dilution into the measuring range for evaluation. Evaluation of linearity, precision and accuracy after dilution of samples was evaluated.

Evaluation of linearity for samples with elevated methotrexate levels:

A sample containing approximately 1200 µmol/L of methotrexate in human serum was prepared to assess the proportional measurement of the drug upon dilution into the calibration range with ARK Methotrexate Dilution Buffer. This HIGH sample was diluted proportionally in pooled human serum to obtain concentrations ranging from 2 to 1200 µmol/L. As a pre-analytical step, these serum samples were diluted in ARK Methotrexate Dilution Buffer. Ten-fold dilution factors were used. Two separately calibrated analytical runs of three replicates of each sample per run were assayed (N=6). Linear regression analysis was performed to demonstrate by visual analysis that measurement of methotrexate was adequately proportional for concentrations exceeding the calibration range.

Measurement of methotrexate is linear within the calibration range of the ARK Methotrexate Assay when the predicted 1st and 2nd order regressed values agree within 10% for concentrations >0.10 µmol/L or within ±0.01 µmol/L at concentrations ≤0.10 µmol/L.

Measurement of elevated methotrexate above the calibration range was linear. Regression analysis demonstrated proportionality along a straight line by

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visual inspection. Reported values (after calculating for dilution) were plotted versus the expected concentration of methotrexate (2.00 to 1200.00 µmol/L).

Expected (µM)	**2.00**	**8.00**	**20.00**	**80.00**	**200.00**	**800.00**	**1200.00**
Dilution	**1/10**	**1/10**	**1/100**	**1/100**	**1/1000**	**1/1000**	**1/10000**
1	0.21	0.75	0.20	0.83	0.20	0.79	0.12
2	0.21	0.76	0.20	0.78	0.20	0.76	0.11
3	0.20	0.82	0.21	0.85	0.20	0.75	0.12
4	0.21	0.75	0.19	0.82	0.20	0.74	0.11
5	0.20	0.78	0.20	0.80	0.20	0.76	0.11
6	0.20	0.79	0.19	0.82	0.19	0.75	0.12
Mean (µM)	**0.205**	**0.775**	**0.198**	**0.817**	**0.198**	**0.758**	**0.115**
SD	**0.005**	**0.027**	**0.008**	**0.024**	**0.004**	**0.017**	**0.005**
Reported (µM)	**2.05**	**7.75**	**19.83**	**81.67**	**198.33**	**758.33**	**1150.00**

Linear regression analysis of this study yielded the following:

$y = 0.9537x + 2.1285$
$R^2 = 0.9999$

Evaluation of precision in samples with elevated methotrexate levels:

Precision was evaluated as described above in 1a.

ARK Methotrexate High Range Controls are comprised of three levels (5, 50, and 500 µmol/L). These controls are comprised in a proteinaceous synthetic matrix equivalent to human serum/plasma.

High Range controls and patient sample pools with concentrations greater than the calibration range were diluted 10x, 100x or 1000x, respectively in ARK Methotrexate Dilution Buffer prior to testing. High Range controls and samples were diluted once for each run and these diluted samples were tested in quadruplicate

Human serum/plasma specimens that contained methotrexate were pooled to create six levels with sufficient volume to complete the 20-day protocol. High patient sample pools (approximately 5, 50 and 500 µmol/L) required that the sample pools were supplemented with methotrexate stock. The stock solution used was prepared by gravimetric addition of pure Methotrexate (USP >99.9 % purity) to dimethylformamide (DMF) and volumetric addition of this stock solution to the specimen pool.

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Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
Controls								
Control 5	160	4.8	0.15	3.1	0.13	2.8	0.20	4.2
Control 50	160	49	1.36	2.8	2.32	4.8	2.72	5.6
Control 500	160	476	15.17	3.2	30.75	6.5	34.66	7.3
Patient Pools								
Patient Pool – 5	160	4.6	0.14	3.1	0.183	4.0	0.24	5.3
Patient Pool – 50	160	45	1.33	3.0	2.63	5.9	2.93	6.6
Patient Pool – 500	160	461	11.84	2.6	27.04	5.9	29.60	6.4

Evaluation of accuracy in samples with elevated methotrexate levels:

A method comparison study was performed to evaluate the agreement between the ARK Methotrexate Assay and predicate device Abbott TDx/TDxFLx Methotrexate II assay (k932615) using samples with elevated methotrexate levels (i.e., samples that require dilution into the assay's measuring range for evaluation). The study was performed using Clinical and Laboratory Standards Institute (CLSI) Protocol EP9-A2 *Method Comparison and Bias Using Patient Samples.*

Forty five patient specimens above the claimed measuring range were evaluated with the proposed and predicate assay; eight samples were spiked with methotrexate. All samples required dilution before testing on both devices (ARK Methotrexate Dilution Buffer was used for the proposed device. The samples ranged from 1.56 – 1440 µmol/L on the predicate. Results of the study are summarized in the table below:

Parameter	Range 1.56 to 1440 µmol/L
Number of Samples	45
Slope	0.96 (0.94 to 0.99)
y-intercept	0.11 (-0.52 to 0.33)
Correlation Coefficient (r^2)	0.997 (0.994 to 0.998)

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

ARK Methotrexate Calibrators and controls are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate.

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ARK Methotrexate Calibrator

A master calibrator lot is prepared volumetrically using a certified solution of methotrexate. The ARK Methotrexate Calibrator is traceable to a certified methotrexate solution and the uncertainty in the methotrexate concentration is 5% relative to the concentration in the certified solution.

The concentration of methotrexate in the certified solution is traceable to HPLC; the purity of methotrexate in the certified solution is determined by HPLC and other spectral procedures as performed by the supplier of the certified solution.

Bulk solutions of the ARK Methotrexate Calibrator are prepared volumetrically using the certified solution of methotrexate. The concentration of methotrexate in the respective bulk solution must agree within 5% of its corresponding master calibrator.

Value Assignment: Testing is performed with the ARK Methotrexate Assay on the Roche/Hitachi 917 automated analyzer. Two calibrated runs are performed using the Master Calibrator. In each run, five replicates of Master Lot (reference) and Test Lot are tested as matched pairs for each calibrator level. Mean values for ten replicates are calculated. Test lot mean values are expected to match the Master lot mean values within 5% allowance. Target values for the six levels of calibrator are 0.00 umol/L, 0.05 umol/L, 0.15 umol/L, 0.25 umol/L, 0.50 umol/L and 1.2 umol/L.

ARK Methotrexate Control

ARK uses USP methotrexate powder to produce the controls. Six levels are provided: three levels (0.07, 0.40 and 0.80 umol/L) are within the calibration range and three levels above the calibration range (5, 50 and 500 µmol/L) are available for controlling the dilution step. ARK manufactures the controls gravimetrically to contain methotrexate within 10% of the target levels.

Value Assignment: Testing is performed with the ARK Methotrexate Assay on the Roche/Hitachi 917 automated analyzer, calibrated with the master calibrator lot. Three calibrated runs are performed using five replicates of each level per run. The 5, 50, 500 µmol/L controls are pre-diluted 1:10, 1:100, and 1:1000 respectively with the ARK Methotrexate Dilution Buffer and tested. Results for high range controls are multiplied by the applicable dilution factor. Mean values (15 replicates) for the test lots are expected to result within 10% of the nominal concentration. The control ranges are set to be +/- 25% from the mean values. Each laboratory should establish the mean value for each control level and its own ranges for each new lot of controls.

Calibrator and Control Stability:

The calibrators and controls are stable until the expiration date printed on the vial when stored unopened and opened at 2-8 °C.

Real time stability studies are ongoing for both unopened and opened

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calibrators and controls. Stability testing protocols and sponsor's acceptance criteria were reviewed and found to be acceptable.

d. Detection limit:

Limit of Detection (LoD) Limit of Quantitation (LoQ) studies were conducted using CLSI Guideline EP 17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation.* The study was performed on the Roche/Hitachi 917 automated clinical chemistry analyzer.

Samples were prepared by gravimetric addition of pure methotrexate (USP) to dimethylformamide and volumetric addition of this stock solution to human serum negative for methotrexate. Pooled human serum representative of the patient specimen matrix was supplemented with methotrexate to give concentrations of 0.02, 0.03, 0.04, and 0.05 µmol/L.

The LoB and LoD were evaluated by testing 60 replicates of pooled human serum (BLANK) and 60 replicates of the first positive level 0.02 µmol/L methotrexate. The analyzer was calibrated and three analytical runs were performed. In each run, 20 replicates of BLANK and 20 replicates of 0.02 µmol/L methotrexate were analyzed. The grand mean and root mean square standard deviation (RMS SD) were calculated. Statistical analyses were performed according to the CLSI Guideline. Since the analyzer did not report values below 0.00 µmol/L, the standard deviation for 0.02 µmol/L methotrexate was used to calculate the LoB based on the equation $LoB = \mu B + 1.645(\sigma B)$, the standard deviation being relatively constant in this area of measurement. The error of the BLANK (σB) was taken to equal the standard deviation of the sample (SDs) as a fair estimate.

The LoQ was determined after testing samples containing 0.03, 0.04, or 0.05 µmol/L. Eight replicates of each sample were tested in each of five runs (each run performed on a separate day) to yield 40 replicates of each LoQ sample tested. The grand mean and RMS SD were calculated for each sample.

The LoQ of the ARK Methotrexate Assay is defined as the lowest concentration for which acceptable inter-assay precision (≤0.01 SD) and recovery (± 0.01 µmol/L) is observed. Where this concentration's 95% confidence interval exceeds the LoD, the definition of LoQ according to the CLSI Guideline will be met. The criteria of LoQ were met at 0.04 µmol/L. (The same LoQ was established when a similar study was performed as above but also incorporating three lots of reagents, controls and calibrators.)

The LoB was determined as 0.01 µmol/L, and the LoD was determined as 0.02 µmol/L using SD = 0.005 µmol/L. LoQ was calculated to be 0.04 µmol/L.

e. Analytical specificity:

Potential interferences of endogenous materials were evaluated according to Clinical and Laboratory Standards Institute (CLSI) Guideline EP7-A2: *Interference Testing in Clinical Chemistry.*

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Clinically high concentrations of potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Pooled human serum supplemented exogenously with endogenous interfering compounds was prepared prior to addition of methotrexate.

Gravimetric addition of methotrexate (USP > 99.9% purity) in dimethyformamide (DMF) and volumetric addition of this stock solution to samples was made to obtain the levels of methotrexate.

Six replicates of each sample and their respective serum controls were tested. Respective runs were co-calibrated with the ARK Methotrexate Calibrator. Tri-level calibration range quality controls of the ARK Methotrexate Control were used to qualify runs. The mean results of methotrexate were calculated and the percentage recoveries relative to the respective serum control mean results were determined.

Interference was defined as follows:

- At methotrexate concentrations near 0.05 µmol/L, the mean concentration of methotrexate in the presence of elevated endogenous substances should fall outside ± 0.02 µmol/L of the mean result for the respective serum control.
- At methotrexate concentrations near mid calibration range (0.50 µmol/L), the mean concentration of methotrexate in the presence of elevated endogenous substances should result in > 10% error in detecting methotrexate in comparison to the mean result for the respective serum control.

Interfering Substance	Interferent Concentration
Albumin	12 g/dL
Bilirubin - conjugated	70 mg/dL
Bilirubin - unconjugated	70 mg/dL
Cholesterol	400 mg/dL
Gamma-Globulin	12 g/dL
Hemoglobin	1000 mg/dL
Intralipid®	500 mg/dL
Rheumatoid Factor	1100 IU/mL
Triglycerides	749 mg/dL
Uric Acid	30 mg/dL

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Interference with any of these substances was not detected at either level of methotrexate tested.

Methotrexate's metabolites, structurally similar compounds, folate derivatives, and potentially co-administered medications were tested to determine whether these compounds affect the quantitation of methotrexate concentrations using the ARK™ Methotrexate Assay.

7-Hydroxymethotrexate (7-OH-MTX) is the main metabolite in serum following high-dose methotrexate (HDMTX) treatment. The concentration of 7-OH-MTX may exceed that of the parent compound by up to 100-fold in plasma shortly after MTX infusion. Methotrexate is also metabolized by intestinal bacteria to the minor, inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA).

Pooled human serum was supplemented with methotrexate prior to addition of potentially cross reacting metabolites (7-OH-MTX and DAMPA) or other compounds with structural similarity. Preparation of serum pools with 0.05 and 0.50 µmol/L methotrexate were identical to the interference study above. Then the potentially cross reactive compounds at their respectively solvated concentrations were added to serum either in the absence of methotrexate or to serum containing methotrexate. Solvent controls for each potentially cross-reacting interferent sample were also prepared.

The respective concentrations of cross reacting interferents were as recommended by CLSI EP7-A2, or in the case of trimethoprim and triamterene, above therapeutic levels as tested. Six (6) replicates of each sample and their respective solvent serum controls were tested. The mean results of methotrexate were calculated. The percentage cross reactivity was determined for each compound tested.

The ARK Methotrexate Assay did not cross react ($\leq 0.01\%$) with folate analogs or other compounds at $\geq$ 1000 µmol/L as tested:

Compound	Tested (µmol/L)	Compound	Tested (µmol/L)
Adriamycin	1000	5-Methyltetra-hydrofolic acid	1000
Cyclophosphamide	1500	Prednisolone	1000
Cytosine	1000	Pyrimethamine	1000
Dihydrofolic Acid	1000	Sulfamethoxazole	1600
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000	Tetrahydrofolic Acid	1000
Folic Acid	1000	Vinblastine	1000
Folinic Acid (leucovorin)	1000	Vincristine	1000
5-Fluorouracil	3000		
6-Mercaptopurine	1000		

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Cross reactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate Assay did not cross react (≤ 0.07%) with the major metabolite 7-OH-MTX.

Cross reactivity to the minor, inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA)

The ARK Methotrexate Assay cross reacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Cross reactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that cross react

The ARK Methotrexate Assay cross reacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for MTX cancer treatment due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

Compound	Tested (µmol/L)	Methotrexate Absent		Methotrexate Present 0.05 µmol/L		Methotrexate Present 0.50 µmol/L	
		MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)	MTX (µmol/L)	Cross Reactivity (%)
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed using Clinical and Laboratory Standards Institute (CLSI) Protocol EP9-A2 *Method Comparison and Bias Using Patient Samples*. Method comparisons were performed to evaluate the agreement between the ARK Methotrexate Assay and fluorescence polarization immunoassay (FPIA), predicate device Abbott TDx/TDxFLx Methotrexate II assay, k932615.

102 leftover specimens within the claimed measuring range were used for method comparison of the ARK Methotrexate Assay and the FPIA predicate device (TDx Assay). Both serum and plasma specimens were included in the study. Singlicate measurements of all specimens were used for method comparison.

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Ten specimens were created by pooling or supplementation with methotrexate (USP). to contain methotrexate concentrations useful for the method comparison.

Summary of the method comparison data:

Parameter	Range 0.04 to 1.19 µmol/L
Number of Samples	102
Slope	1.00 (1.00 – 1.02)
y-intercept	0.01 (0.00 to 0.01)
Correlation Coefficient (r^2)	0.978 (0.968 to 0.985)

b. *Matrix comparison:*

Anticoagulated plasma and serum were evaluated to demonstrate equivalency of these matrices for measurement of methotrexate with the ARK™ Methotrexate Assay. Matched samples for serum and plasma from eight subjects were evaluated.

In this study, blood was separated into plasma or serum prior to supplementation with methotrexate. Each subject donated in three different anticoagulant tubes and a serum tube to produce a matched set. A methotrexate stock solution was prepared by gravimetric addition of methotrexate (USP > 99% purity) to dimethylformamide (DMF). For each matrix and subject, an aliquot was supplemented by volumetric addition of methotrexate stock solution.

For eight subjects, methotrexate was added to each matrix to give 1.00 µmol/L, and then serial two-fold dilutions were made with its own matrix/subject to give 0.50 and 0.25 µmol/L, respectively.

For four subjects, methotrexate was added to each matrix to give 0.05 µmol/L.

Since the calibration range spans 0.00 to 1.20 µmol/L, equivalency of serum and plasma matrices for measurement of methotrexate with the ARK Methotrexate Assay was evaluated from near the lower limit of quantitation (0.04 µmol/L) to the upper calibration range.

Compared to the mean methotrexate level in the individual's matched serum control, the mean methotrexate concentration in the individual's corresponding plasma was acceptable if the concentration measured was within ±10% of the serum control for concentrations at > 0.1 µmol/L and within ±0.02 µmol/L at methotrexate concentrations ≤ 0.1 µmol/L.

The overall percentage of serum levels of spiked methotrexate above 0.10 µmol/L in plasma ranged 93.7 to 107.4%. At 0.05 µmol/L methotrexate, the difference in measurement of methotrexate between the plasma samples and serum controls was ≤0.01 µmol/L.

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3. Clinical studies:

   a. *Clinical Sensitivity:*

      Not applicable. Not typical for this type of assay.

   b. *Clinical specificity:*

      Not applicable. Not typical for this type of assay.

   c. Other clinical supportive data (when a. and b. are not applicable):

      The sponsor provided a discussion with balanced and representative literature discussing clinical use of methotrexate measurements.

4. Clinical cut-off:

   See expected values below.

5. Expected values/Reference range:

   The following is included in the package insert:

   Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors.[1,2] While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example),[6] concentrations may exceed 1000 µmol/L[7] with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.[8] For treatment of osteosarcoma,[7] the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, less than 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin in accordance with algorithms until the MTX level is <0.1 µmol/L. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.[1,3] Some centers follow ≤ 0.10 µmol/L.[7,9]

   From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.[1,3,10]

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Clinical Situation	Laboratory Findings	
	Methotrexate Level (μmol/L)	Hours after administration
Normal Methotrexate Elimination	~10	24
	~1	48
	<0.2	72
Delayed Late Methotrexate Elimination	>0.2	72
	>0.05	96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50	24
	≥5	48
	OR	
	≥100% increase in serum creatinine	24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs,[5, 10] for example vancomycin.[11] Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), hematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.[12-19]

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite,[6, 18] its molar ratio to methotrexate of up to approximately 100-fold,[20] and relative insolubility versus the parent drug,[5, 10] possible nephrotoxicity due to precipitation of the metabolite in renal tubules[20] may delay elimination of methotrexate itself.

Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.[5] Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.[4]

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14. Chessells, J. M. et al. 1990. Neurotoxicity in lymphoblastic leukaemia: Comparison of oral and intramuscular methotrexate and two doses of radiation. *Archives of Disease in Childhood* 65:416 – 422.

15. Allen, J. C. et al. 1980. Leukoencephalopathy following high-dose IV methotrexate chemotherapy with leucovorin rescue. *Cancer Treat Rep* 64:1261 – 1273.

16. Jacobs, P. et al. 1991. Methotrexate encephalopathy. *Eur J Cancer* 27:1061 – 1062.

17. Flombaum, C. D. and Meyers, P. A. 1999. High-Dose Leucovorin as Sole Therapy for Methotrexate Toxicity. *J Clin Oncol* 17:1589 – 1594.

18. Collier, C. P. et al. 1982. Analysis of methotrexate and 7-hydroxymethotrexate by high-performance liquid chromatography and preliminary clinical studies. *Ther Drug Monit* 4:371 – 380.

19. Widemann, B. C. et al. 2010. Glucarpidase, leucovorin, and thymidine for high-dose methotrexate-induced renal dysfunction: clinical and pharmacologic factors affecting outcome. *J Clin Oncol* 28:3979 – 3986.

20. Erttmann, R. et al. 1985. 7-Hydroxy-Methotrexate and Clinical Toxicity Following High-Dose Methotrexate Therapy. *J Cancer Res Clin Oncol* 109:86 – 88.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

P. Other Supportive Device and Instrument Information:

The post-market database was searched, and there were no issues reported under these product codes (LAO, DLJ, LAS) for this manufacturer.

Q. Administrative Information:

1. Applicant contact information:

   a. Name of applicant:

   ARK Diagnostics, Inc.

   b. Mailing address:

   1190 Bordeaux Dr.

   Sunnyvale, CA 94089

   c. Phone #:

   408-747-0700

   d. Fax #:

   408-747-0783

   e. E-mail address (optional):

   f. Contact:

   Kenneth C. Kasper, PhD, Executive Director of Quality and Regulatory Affairs

2. Review documentation:

   Submission received July 5, 2011. After preliminary review of this submission, it was placed on hold on 8/17/11 because this submission had deficiencies in method comparison, limit of detection/limit of Quantitation, linearity and matrix comparison. A response was received on September 7, 2011. The applicant has adequately answered all deficiencies identified.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

3. Substantial Equivalence Discussion:

	Yes	No	
1. Same Indication Statement?	X		If **YES** = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?		X	If **YES** = Stop **NSE**
3. Same Technological Characteristics?	X		If **YES** = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 6
5. Descriptive Characteristics Precise Enough?		X	If **NO** = Go To 8 If **YES** = Stop **SE**
6. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NSE**
7. Accepted Scientific Methods Exist?			If **NO** = Stop **NSE**
8. Performance Data Available?	X		If **NO** = Request Data
9. Data Demonstrate Equivalence?	X		Final Decision: CS

Note: See http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_4148/FLOWCHART%20DECISION%20TREE%20.DOC for Flowchart to assist in decision-making process. Please complete the following table and answer the corresponding questions. "Yes" responses to questions 2, 4, 6, and 9, and every "no" response requires an explanation.

a. *Explain how the new indication differs from the predicate device's indication:*

b. *Explain why there is or is not a new effect or safety or effectiveness issue:*

c. *Describe the new technological characteristics:*

d. *Explain how new characteristics could or could not affect safety or effectiveness:*

e. *Explain how descriptive characteristics are not precise enough:*
Because the clinical use of this *in vitro* diagnostic device is based upon its performance characteristics, descriptive characteristics alone are not sufficient to support a claim of substantial equivalence to marketed *in vitro* diagnostic devices.

f. *Explain new types of safety or effectiveness question(s) raised or why the question(s) are not new:*

g. *Explain why existing scientific methods can not be used:*

h. *Explain what performance data is needed:*

i. *Explain how the performance data demonstrates that the device is or is not substantially equivalent:*

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Because the performance data are customary for an *in vitro* diagnostic device for this intended use, these data demonstrate that the device is substantially equivalent. See decision summary template in section M.2.a. above for more information.

R. Reviewer Name and Signature:

Kellie B. Kelm, Ph.D
CDRH/OIVD/DCTD

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Product Code	**DLJ**
Class	**2**
Regulation Device Classification	**Clinical toxicology calibrator.**
Device Name	**CALIBRATORS, DRUG SPECIFIC**
Date Created	**1/1/1990**
Performance Standard #	

CDRH Gen Docs (None)

Premarket Reviews (20)

Manufacturer	Type	Total	Approved	Denied	Other	Pending
ABBOT LABORATORIES	510(k)	2	2			
ABBOTT LABORATORIES INC	510(k)	1	1			
ARK DIAGNOSTICS,INC	510(k)	6	5			1
BEDFONT SCIENTIFIC LTD.	510(k)	1	1			
BIOKIT S.A.	510(k)	2	2			
DADE BEHRING, INC.	510(k)	1			1	
DIAGNOSTICA STAGO	510(k)	1			1	
DIAGNOSTICO STAGO SAS	510(k)	1			1	
FUJIREBIO DIAGNOSTICS, INC.	510(k)	1	1			
IMMUNALYSIS CORPORATION	510(k)	5			5	
[redacted]ZHI INTERNATIONAL, INC.	510(k)	5	5			
[redacted]ROGENICS CORP.	510(k)	3	3			
MICROGENICS CORPORATION	510(k)	1				1
NOVX SYSTEMS INC.	510(k)	1	1			
RANDOX LABORATORIES, LTD.	510(k)	4	4			
SIEMENS HEALTHCARE DIAGNOSTICS	510(k)	2	2			
SIEMENS HEALTHCARE DIAGNOSTICS INC.	510(k)	2	2			
SIEMENS HEATLHCARE DIAGNOSTICS INC	510(k)	1	1			
SIEMENS MEDICAL SOLUTIONS DIAGNOSTICS	510(k)	1	1			
THERMO FISHER SCIENTIFIC	510(k)	3	2		1	

Standards and Guidance (None)

MDR Summary (1)

	2011	Total
MALFUNCTION	1	1
Total	1	1

MDR distribution by manufacturer - Death or Injury ()

[redacted]tient Problems (1)

[redacted]ient Problem Description	Total	Malfunction
No known impact or consequence to patient	1	1

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Device Problems (3)

ice Problem Description	Total	Malfunction
Cap	1	1
Leak	1	1
Slippage of device or device component	1	1

Recalls (1)

	Class II	Total
BIOKIT SA	1	1
Total	1	1

Inspections and EIRs (2 manufacturers)

Manufacturer	Inspections			EIRs		
BECKMAN COULTER, INC.	0	0	0	0	0	0
BIOKIT SA	2	0	2	0	1	0
	2	**0**	**2**	**0**	**1**	**0**

Radiation Safety (None)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Product Code	**LAO**
Class	**Unclassified**
Regulation Device Classification	
Device Name	**ENZYME IMMUNOASSAY, METHOTREXATE**
Date Created	**12/5/1996**
Performance Standard #	

CDRH Gen Docs (None)

Premarket Reviews (1)

Manufacturer	Type	Total	Approved	Denied	Other	Pending
ARK DIAGNOSTICS,INC	510(k)	1				1

Standards and Guidance (None)

MDR Summary (1)

	2009	Total
INJURY	1	1
Total	1	1

MDR distribution by manufacturer - Death or Injury (1)

ufacturer	Total
BBOTT DIAGNOSTICS INTERNATIONAL, LTD.	1

Patient Problems (1)

Patient Problem Description	Total	Injury
Overdose	1	1

Device Problems (1)

Device Problem Description	Total	Injury
Use of Device Issue	1	1

Recalls (None)

Inspections and EIRs (1 manufacturers)

Manufacturer	Inspections			EIRs		
ABBOTT DIAGNOSTICS INTERNATIONAL, LTD.	0	0	0	0	0	0
	0	0	0	0	0	0

Radiation Safety (None)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Product Code	**LAS**
Class	**1**
Regulation Device Classification	**Clinical toxicology control material.**
Device Name	**DRUG SPECIFIC CONTROL MATERIALS**
Date Created	**1/1/1990**
Performance Standard #	

CDRH Gen Docs (None)

Premarket Reviews (9)

Manufacturer	Type	Total	Approved	Denied	Other	Pending
ARK DIAGNOSTICS,INC	510(k)	6	5			1
BEDFONT SCIENTIFIC LTD.	510(k)	1	1			
CHROMSYSTEMS INSTRUMENTS & CHEMICALS GMBH	510(k)	1	1			
IMMUNALYSIS CORPORATION	510(k)	5			5	
LIN-ZHI INTERNATIONAL, INC.	510(k)	5	5			
MICROGENICS CORP.	510(k)	1	1			
RANDOX LABORATORIES, LTD.	510(k)	4	4			
THERMO FISHER SCIENTIFIC	510(k)	2	1		1	
UNKNOWN	510(k)	1	1			

ndards and Guidance (None)

MDR Summary (None)

MDR distribution by manufacturer - Death or Injury (None)

Patient Problems (None)

Device Problems (None)

Recalls (None)

Inspections and EIRs (None)

Radiation Safety (None)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Product Code	Class	Regulation	Device Name	Date Created

Registration and Listing

Premarket Reviews Completed (None)

Under Review, Withdrawn or Closed without Product Code (1)

K111904	ARK METHOTREXATE ASSAY, ARK METHOTREXATE CALIBRATOR, ARK METHOTREXATE CONTROL, ARK METHOTREXATE CONTROL (CALIBRATION RAN	Review	Kellie Kelm
Image	FDA.GOV		K932615

MDR Summary (None)

MDR Analyst (None)

MDR Distribution by Brand - Death or Injury (None)

Patient Problems (None)

Patient Outcomes (None)

Device Problems (None)

Manufacturer Evaluation Results (None)

Manufacturer Evaluation Conclusions (None)

Recalls (None)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Kelm, Kellie

From: Ken Kasper [ken@ark-tdm.com]
Sent: Friday, October 14, 2011 2:23 PM
To: Kelm,'Kellie
Subject: Re: k111904 ARK (b)(4)
Attachments: (b)(4) df
)

Hi Kellie,

(b)(4)

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Friday, October 14, 2011 10:14 AM
Subject: RE: k111904 ARK (b)(4)

Ken,

(b)(4)

Kellie

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Friday, October 14, 2011 12:23 PM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Hi Kellie,

(b)(4) Deficiencies

[redacted]

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Friday, October 14, 2011 5:53 AM
Subject: RE: k111904 ARK (b)(4) [redacted]

Ken,

(b)(4)

[redacted]

Regards,
Kellie Kelm

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Thursday, October 13, 2011 3:55 PM

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4)

Hi Kellie,

(b)(4)

(b)(4)

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Thursday, October 13, 2011 11:58 AM
Subject: RE: k111904 ARK (b)(4)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Ken,

(b)(4) [redacted]

Regards,
Kellie Kelm

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Thursday, October 13, 2011 2:23 PM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4) [redacted]

Hello Kellie,

(b)(4) [redacted]

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Thursday, October 13, 2011 6:54 AM
Subject: RE: k111904 ARK (b)(4) [redacted]

Ken,

I met with my Deputy Director to discuss (b)(4) [redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4) [redacted]

Regards,
Kellie Kelm

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Wednesday, October 12, 2011 2:05 PM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4) [redacted]

Hi Kellie,

(b)(4) [redacted]

Thanks, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Friday, October 7, 2011 1:30 PM
Subject: RE: k111904([redacted]
b

Ken,

(b)(4) [redacted]

We can talk on Wednesday when you return.

Kellie

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Friday, October 07, 2011 5:39 AM
To: Kelm, Kellie
Subject: Re: k111904 (b)(4)

Dear Kellie,

(b)(4)

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>
To: Ken Kasper <ken@ark-tdm.com>
Sent: Thursday, October 6, 2011 4:16 PM
Subject: RE: k111904 ARK (b)(4)

Ken,

(b)(4)

38

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(b)(4)

[redacted]

Thanks,
Kellie Kelm

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Thursday, October 06, 2011 6:58 AM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4)

Dear Kellie,

(b)(4)

[redacted]

Best regards, Ken

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089

phone 408-747-0708
fax 408-747-0783

From: "Kelm, Kellie" <Kellie.Kelm@fda.hhs.gov>

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

To: Ken Kasper <ken@ark-tdm.com>
Sent: Wednesday, October 5, 2011 9:38 PM
Subject: RE: k111904 ARK(b)(4)

Ken,

(b)(4)

Thank you in advance.

Regards,
Kellie Kelm
301-796-6145 - phone
* Guidance for Industry and FDA Staff - Assayed and Unassayed Quality Control Material:
http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079179.htm

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

DRAFT

ARK™ *Methotrexate Control*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Control must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

© 2011, ARK Diagnostics, Inc. **Control Kit REF 5026-0003-00**

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

1 Name

ARK™ *Methotrexate Control*

2 Intended Use

ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Control is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description	Quality Control
5026-0003-00 Complete Set	**ARK Methotrexate Control* (2 mL)** Methotrexate, buffer, bovine serum albumin, and preservatives (nominal level)	**Expected Range (µmol/L)****
5026-0003-01 Calibration Range Controls[1]	**LOW** (0.07 µmol/L)	0.05 – 0.09
	MID (0.40 µmol/L)	0.30 – 0.50
	HIGH (0.80 µmol/L)	0.60 – 1.00
5026-0003-02 High Range Controls[2]	**5** µmol/L	3.75 – 6.25
	50 µmol/L	37.50 – 62.50
	500 µmol/L	375.00 – 625.00

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0318, 0.1818, 0.3636, 2.272, 22.72, and 227.2 µg/mL respectively.

**The ARK Methotrexate Control is a precision control. Each laboratory should establish the mean value for each control level and its own ranges for each new lot of controls.

[1] Calibration range controls may be obtained separately as a set, REF 5026-0003-01.

[2] High range controls may be obtained separately as a set, REF 5026-0003-02.

4 Warnings and Precautions

- For ***In Vitro* Diagnostic** Use.
- Do not mix controls from different lot numbers.
- Use each lot as a set.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

5 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Controls are ready to use. Mix each level by gentle inversion before dispensing.
- High range controls must be diluted prior to testing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

6 Limitations of Procedure

All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

7 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0215-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

DRAFT

ARK™ *Methotrexate Calibrator*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Calibrator must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	Calibrator	Calibrator

© 2011, ARK Diagnostics, Inc. **Calibrator Kit REF 5026-0002-00**

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

1 Name

ARK™ *Methotrexate Calibrator*

2 Intended Use

ARK Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Calibrator is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description		Quantity/Volume
5026-0002-00	**ARK Methotrexate Calibrators*** Methotrexate, buffer, bovine serum albumin, and preservatives		Dropper vials
	A	0.00 µmol/L	1 X 2 mL
	B	0.05 µmol/L	1 X 2 mL
	C	0.15 µmol/L	1 X 2 mL
	D	0.25 µmol/L	1 X 2 mL
	E	0.50 µmol/L	1 X 2 mL
	F	1.20 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0227, 0.0682, 0.1136, 0.2272, and 0.5453 µg/mL for Calibrators B to F respectively.

4 Standardization

There is no internationally recognized standard for Methotrexate. ARK Methotrexate Calibrators are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate.

5 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use.
- Do not mix calibrators from different lot numbers.
- Use each lot as a set.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

6 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Calibrators are ready to use. Mix each level by gentle inversion before dispensing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

7 Procedure

Calibration
Perform a full calibration (6- point) procedure; test calibrators in duplicate. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

8 Limitations of Procedure

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

9 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0214-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

ARK Diagnostics, Inc.

DRAFT

ARK™ *Methotrexate Dilution Buffer*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Dilution Buffer must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

© 2011, ARK Diagnostics, Inc.

Dilution Buffer Kit REF 5026-0004-00

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

1 Name

ARK™ *Methotrexate Dilution Buffer*

2 Intended Use

ARK Methotrexate Dilution Buffer is intended for dilution of specimens containing high concentrations of methotrexate for the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Dilution Buffer is comprised of a synthetic protein matrix. Its composition is equivalent to Calibrator A (zero), ARK Methotrexate Calibrator, REF 5026-0002-00.

REF	Product Description	Quantity/Volume
5026-0004-00	**ARK Methotrexate Dilution Buffer** Buffer, bovine serum albumin, and preservatives	**1 X 25 mL**

4 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use.

5 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Dilution Buffer is ready to use. Mix by gentle inversion before dispensing.
- Store at 2-8°C. Use prior to the expiration date. Once opened, store at 2-8°C for 12 months within the expiration date.

6 Procedure – Manual Dilution Protocol

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the measurement range.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Manually dilute the high specimen or high range control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

Sample Volume		Dilution Matrix Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor. To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

7 Trademarks

ARK™ is a trademark of ARK Diagnostics, Inc.
Other brand or product names are trademarks of their respective holders.

ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised October 2011
1600-0216-00 Rev 02

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Fife, Elizabeth *

From: Microsoft Exchange
To: 'ken@ark-tdm.com'
Sent: Thursday, August 18, 2011 11:14 AM
Subject: Relayed: K111904 Holding

Delivery to these recipients or distribution lists is complete, but delivery notification was not sent by the destination:

'ken@ark-tdm.com'

Subject: K111904 Holding

Sent by Microsoft Exchange Server 2007

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Food and Drug Administration
Office of Device Evaluation &
Office of In Vitro Diagnostics

COVER SHEET MEMORANDUM

From: Reviewer Name Kellie Kelm

Subject: 510(k) Number K111904

To: The Record

Please list CTS decision code TH

☐ Refused to accept (Note: this is considered the first review cycle, See Screening Checklist http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_5631/Screening%20Checklist%207%202%2007.doc)

☒ Hold (Additional Information or Telephone Hold). emailed 8/17/11

☐ Final Decision (SE, SE with Limitations, NSE (select code below), Withdrawn, etc.).

Not Substantially Equivalent (NSE) Codes

☐	NO	NSE for lack of predicate
☐	NI	NSE for new intended use
☐	NQ	NSE for new technology that raises new questions of safety and effectiveness
☐	NP	NSE for lack of performance data
☐	NC	NSE call for PMAs
☐	NS	NSE no response
☐	NH	NSE for another reason

Please complete the following for a final clearance decision (i.e., SE, SE with Limitations, etc.):		YES	NO
Indications for Use Page	*Attach IFU*		
510(k) Summary /510(k) Statement	*Attach Summary*		
Truthful and Accurate Statement.	*Must be present for a Final Decision*		
Is the device Class III?			
If yes, does firm include Class III Summary?	*Must be present for a Final Decision*		
Does firm reference standards? (If yes, please attach form from http://www.fda.gov/opacom/morechoices/fdaforms/FDA-3654.pdf)			
Is this a combination product? (Please specify category _____, see http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_413b/COMBINATION%20PRODUCT%20ALGORITHM%20(REVISED%203-12-03).DOC			
Is this a reprocessed single use device? (Guidance for Industry and FDA Staff – MDUFMA - Validation Data in 510(k)s for Reprocessed Single-Use Medical Devices, http://www.fda.gov/cdrh/ode/guidance/1216.html)			
Is this device intended for pediatric use only?			
Is this a prescription device? (If both prescription & OTC, check both boxes.)			
Did the application include a completed FORM FDA 3674, Certification with Requirements of ClinicalTrials.gov Data Bank?			
Is clinical data necessary to support the review of this 510(k)?			
Did the application include a completed FORM FDA 3674, *Certification with Requirements of ClinicalTrials.gov Data Bank*? (If not, then applicant must be contacted to obtain completed form.)			
Does this device include an Animal Tissue Source?			
All Pediatric Patients age<=21			

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Neonate/Newborn (Birth to 28 days)

Infant (29 days -< 2 years old)

Child (2 years -< 12 years old)

Adolescent (12 years -< 18 years old)

Transitional Adolescent A (18 - <21 years old) Special considerations are being given to this group, different from adults age ≥ 21 (different device design or testing, different protocol procedures, etc.)

Transitional Adolescent B (18 -<= 21; No special considerations compared to adults => 21 years old)

Nanotechnology

Is this device subject to the Tracking Regulation? (Medical Device Tracking Guidance, http://www.fda.gov/cdrh/comp/guidance/169.html) *Contact OC.*

Regulation Number **Class*** **Product Code**

(*If unclassified, see 510(k) Staff)

Additional Product Codes:

Review: (Branch Chief) DCTD (Branch Code) 8/17/2011 (Date)

Final Review: (Division Director) (Date)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

MEMORANDUM

To: "The Record" – k111904 ARK Diagnostics Methotrexate Assay

From: Kellie Kelm, lead reviewer

Date: August 17, 2011

Re: Telephone hold recommendation

I recommend that this 510(k) submission be put on telephone hold. I have identified the deficiencies in the submission to be communicated to the sponsor:

Dear Dr. Kasper:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. We cannot determine if the device is substantially equivalent to a legally marketed predicate device based solely on the information you provided. To complete the review of your submission, we request the following:

(b)(4) Deficiencies

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) Deficiencies

(b)(4) Deficiencies

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Kelm, Kellie

m: Kelm, Kellie
nt: Wednesday, August 17, 2011 2:00 PM
To: 'Ken Kasper'
Subject: k111904 Telephone hold

Dear Dr. Kasper:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. We cannot determine if the device is substantially equivalent to a legally marketed predicate device based solely on the information you provided. To complete the review of your submission, we request the following:

(b)(4) Deficiencies

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) Deficiencies

(b)(4) Deficiencies

[redacted]

The deficiencies identified above represent the issues that we believe need to be resolved before our review of your 510(k) submission can be successfully completed.

If the information, or a request for an extension of time, is not received within 30 days, we will consider your premarket notification to be withdrawn and your submission will be deleted from our system. If you submit the requested information after 30 days it will be considered and processed as a new 510(k)(21 CFR 807.87(l)); therefore, all information previously submitted must be resubmitted so that your new 510(k) is complete. For guidance on 510(k) actions, please see our guidance document entitled, "Guidance for Industry and FDA Staff: Interactive Review for Medical Device Submissions: 510(k)s, Original PMAs, PMA Supplements, Original BLAs, and BLA Supplements" at www.fda.gov/cdrh/ode/guidance/1655.html.

If the submitter does submit a written request for an extension, FDA will permit the 510(k) to remain on hold for up to a maximum of 180 days from the date of the additional information request.

The requested information, or a request for an extension of time, should reference your above 510(k) number and should be submitted in duplicate to:

U.S. Food and Drug Administration
Center for Devices and Radiological Heath
Document Mail Center – WO66-0609
10903 New Hampshire Avenue
Silver Spring, MD 20993

If you have any questions concerning the contents of the letter, please contact Kellie Kelm at (301) 796-6145.

Regards,
Kellie B. Kelm, Ph.D
FDA/CDRH/OIVD/DCTD

[redacted]

Food and Drug Administration
10903 New Hampshire Avenue
WO66, Room 5648

132

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016
Silver Spring, MD 20993-0002

301-796-6145 - phone

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) Draft Document

(b)(4) Draft Document

(b)(4) Draft Document

(b)(4) Draft Document

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Kelm, Kellie

From: Ken Kasper [ken@ark-tdm.com]
Sent: Tuesday, August 09, 2011 3:04 PM
To: Kelm, Kellie
Subject: RE: k111904 ARK (b)(4)

Thanks Kellie for the quick response.

(b)(4)

Thank you again. Best regards, Ken

Best regards, Ken

Kenneth C. Kasper, PhD Executive Director, Quality and Regulatory Affairs ARK Diagnostics Inc.
1190 Bordeaux Drive Sunnyvale, CA 94089 phone 408-747-0708 fax 408-747-0783

--- On **Tue, 8/9/11, Kelm, Kellie <*Kellie.Kelm@fda.hhs.gov*>** wrote:

> From: Kelm, Kellie <Kellie.Kelm@fda.hhs.gov>
> Subject: RE: k111904 ARK (b)(4)
> To: "Ken Kasper" <ken@ark-tdm.com>
> Date: Tuesday, August 9, 2011, 11:04 AM
>
> Ken,
>
> (b)(4)
>
> Regards,
> Kellie

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

From: Ken Kasper [mailto:ken@ark-tdm.com]
Sent: Tuesday, August 09, 2011 1:55 PM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4)

Dear Dr. Kelm,

(b)(4)

Thanks in advance for your response. Best regards, Ken

Kenneth C. Kasper, PhD Executive Director, Quality and Regulatory Affairs ARK Diagnostics Inc. 1190 Bordeaux Drive Sunnyvale, CA 94089 phone 408-747-0708 fax 408-747-0783

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Therapeutic Drug Monitoring
23:259–262 © 2001 Lippincott Williams & Wilkins, Inc., Philadelphia

Stability of Therapeutic Drugs in Serum Collected in Vacutainer Serum Separator Tubes Containing a New Gel (SST II)

Valerie Bush,* John Blennerhasset,† Alice Wells,‡ and Amitava Dasgupta‡

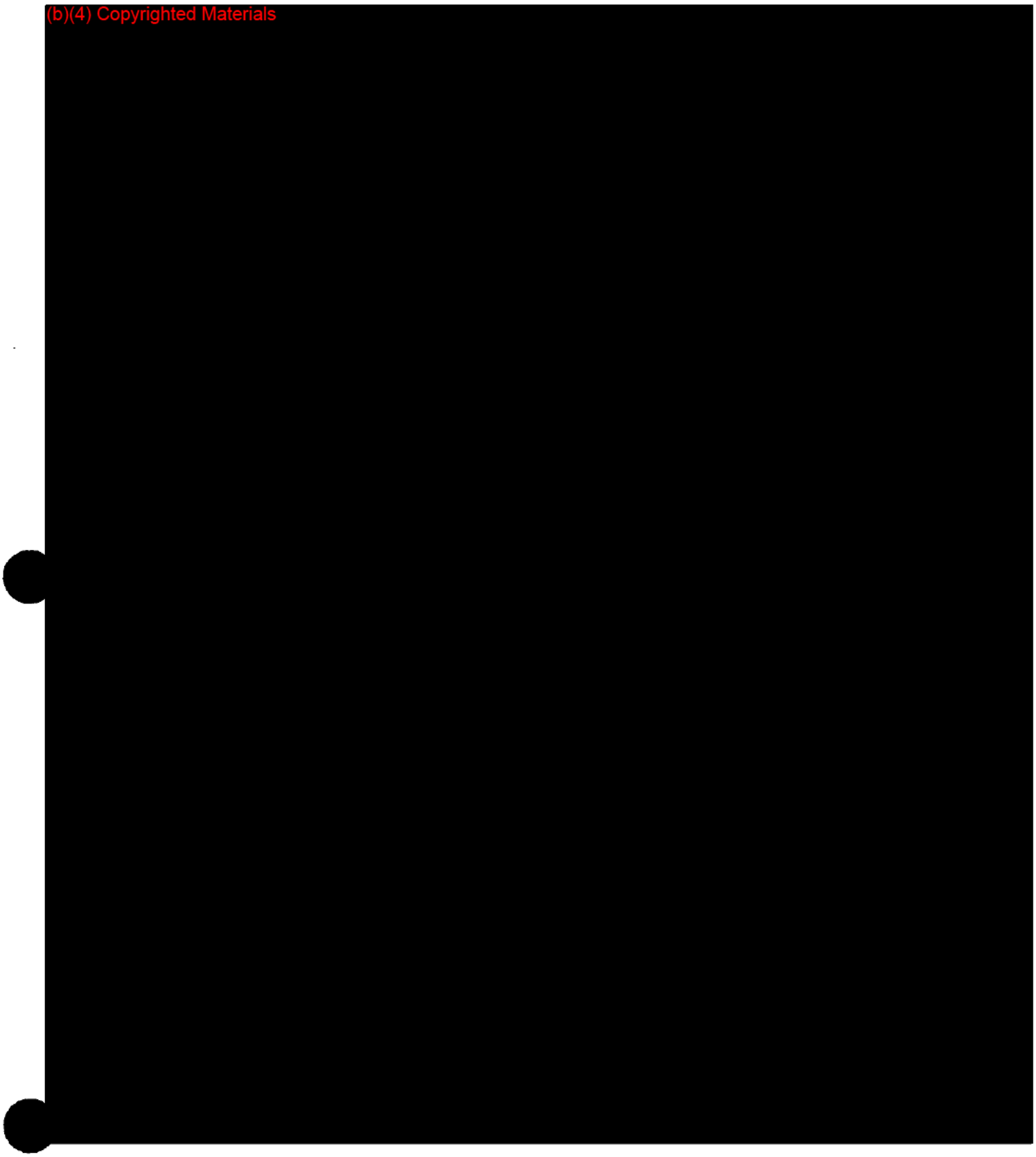
**Becton-Dickinson, Franklin Lake, New Jersey; †Royal Perth Hospital, Perth, Australia; and ‡Department of Pathology and Laboratory Medicine, University of Texas–Houston Medical School, Houston, Texas*

(b)(4) Copyrighted Materials

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

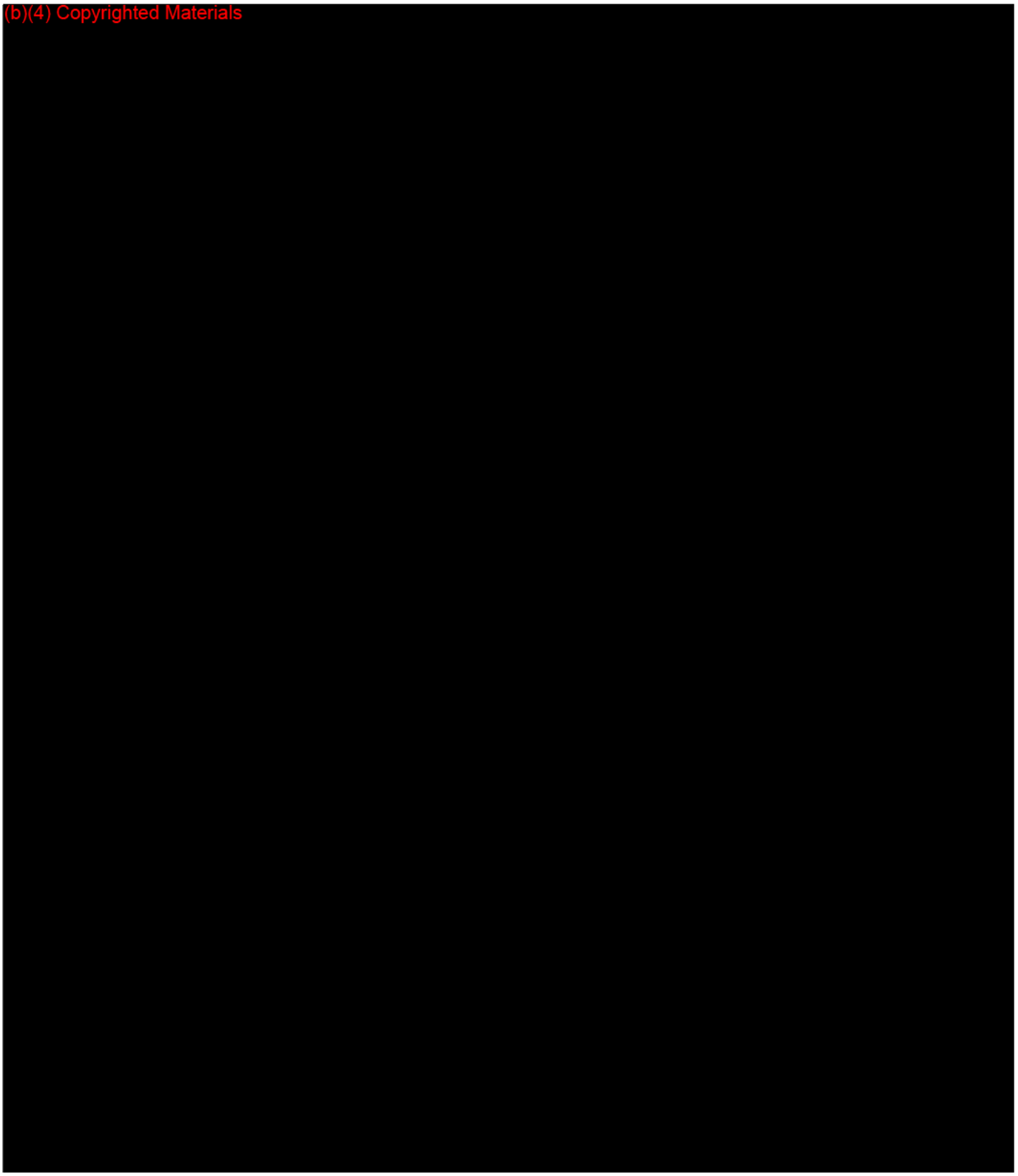
(b)(4) Copyrighted Materials



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

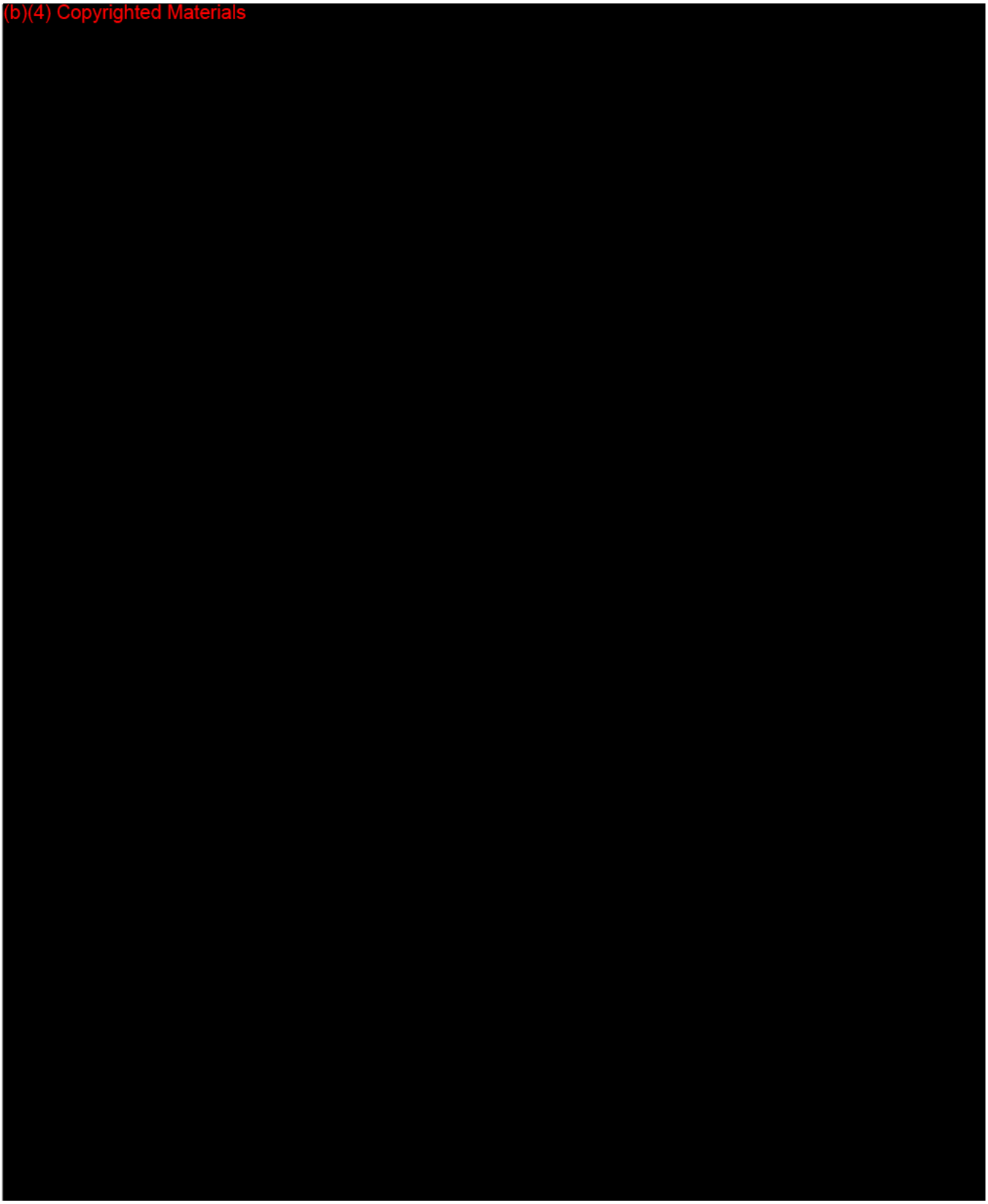
Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4) Copyrighted Materials



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Kelm, Kellie

From: Cross Jr, Frank H
Sent: Wednesday, July 27, 2011 11:46 AM
To: Kelm, Kellie
Cc: Weeks, Dominic*
Subject: DDOP ARK MTX CDRH consult.doc
Attachments: 8-11-11 DDOP ARK MTX CDRH consult.pdf

Hi Kellie,

Attached is out finalized consult.

Please let me know when you receive this.

Frank
Frank H. Cross, Jr., MA, MT (ASCP)
Captain, USPHS Commissioned Corps
Chief, Project Management Staff
Division of Drug Oncology Products
Office of Oncology Drug Products
Center for Drug Evaluation and Research
US Food and Drug Administration
White Oak Bldg 22, Room 2110
10903 New Hampshire Avenue
Silver Spring, MD 20993
(301) 796-0876 (office)
(301) 796-9845 (fax)
frank.crossjr@fda.hhs.gov

Dominic,

Please close consult in our e-room, print this review and e-mail and place in a labeled paper folder for manual filing. Thanks

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Clinical Consultation

FROM: Paul G. Kluetz, M.D.
Medical Reviewer
Division of Drug Oncology Products
OODP/OND/CDER/FDA

Ke Liu, M.D., PhD
Medical Team Leader
Division of Drug Oncology Products
OODP/OND/CDER/FDA

TO: Kellie Kelm
CDRH/OIVD
White Oak Bldg 66, Room 5648

SUBJECT: Clinical Review of ARK Methotrexate Assay

Drug Name(s): Methotrexate (generic)

Methotrexate LPF: 11-719

Applicant/sponsor: Various (Methotrexate), ARK Diagnostics, Inc. (ARK Methotrexate Assay)

DATE CONSULT RECEIVED: July 19, 2011
DESIRED COMPLETION DATE: August 11, 2011
DATE CONSULT COMPLETED: August , 2011

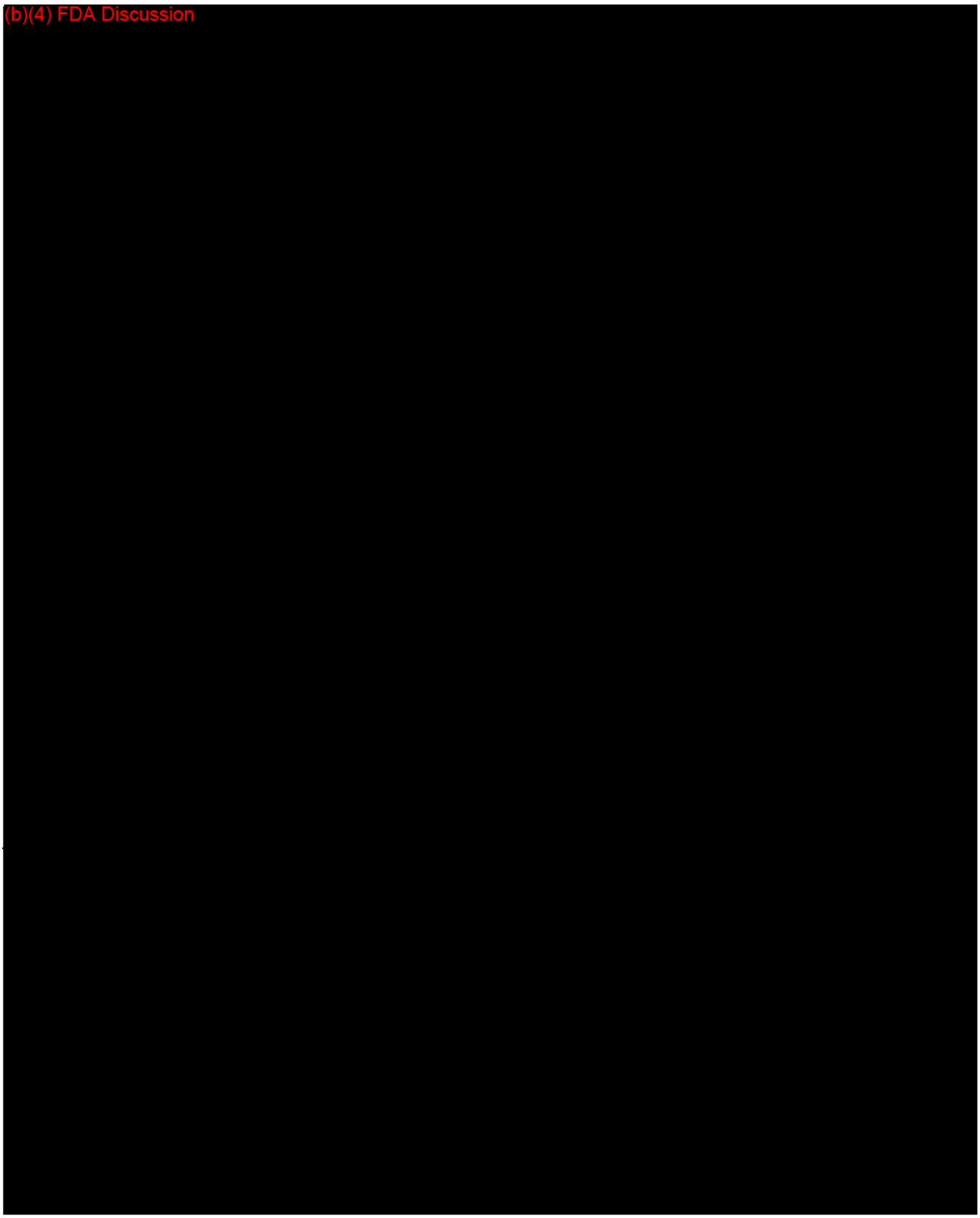
MATERIAL RECEIVED FOR REVIEW

(b)(4)

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016



Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) FDA Discussion

(b)(4) FDA Discussion

(b)(4) FDA Discussion

(b)(4) FDA Discussion

(b)(4) FDA Discussion

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

Kelm, Kellie

From: Ken Kasper [ken@ark-tdm.com]
Sent: Tuesday, July 26, 2011 1:28 PM
To: Kelm, Kellie
Subject: Re: k111904 ARK (b)(4)
Attachments: (b)(4)

Dear Dr. Kelm,

(b)(4)

The additional information you requested is provided in the attached files.

Best regards, Ken

Kenneth C. Kasper, PhD Executive Director, Quality and Regulatory Affairs ARK Diagnostics Inc. 1190 Bordeaux Drive Sunnyvale, CA 94089 phone 408-747-0708 fax 408-747-0783

--- On **Mon, 7/25/11, Kelm, Kellie** <*Kellie.Kelm@fda.hhs.gov*> wrote:

From: Kelm, Kellie <Kellie.Kelm@fda.hhs.gov>
Subject: k111904 ARK (b)(4) ay
To: "ken@ark-tdm.com" <ken@ark-tdm.com>
Date: Monday, July 25, 2011, 12:56 PM

Dear Dr. Kasper,

(b)(4)

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4)

[redacted]

I may have additional questions after further review of your submission.

Thank you in advance.

Regards,
Kellie B. Kelm, Ph.D
FDA/CDRH/OIVD/DCTD

Food and Drug Administration
10903 New Hampshire Avenue
WO66, Room 5648
Silver Spring, MD 20993-0002

301-796-6145 - phone
301-847-8513 - fax

kellie.kelm@fda.hhs.gov

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

July 26, 2011

Kellie B. Kelm, Ph.D.
FDA/CDRH/OIVD/DCTD
Food and Drug Administration
10903 New Hampshire Avenue
WO66, Room 5648
Silver Spring, MD 20993-0002

301-796-6145 - phone
301-847-8513 - fax
kellie.kelm@fda.hhs.gov

RE: 510(K) Premarket Notification K111904, ARK™ Methotrexate Assay

Dear Dr. Kelm,

ARK Diagnostics has received your e-mail dated July 25, 2011 regarding the submission "K111904 ARK Methotrexate Assay". You requested the following additional information.

(b)(4) Deficiencies

[redacted]

154

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4) Deficiencies

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4) Deficiencies

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

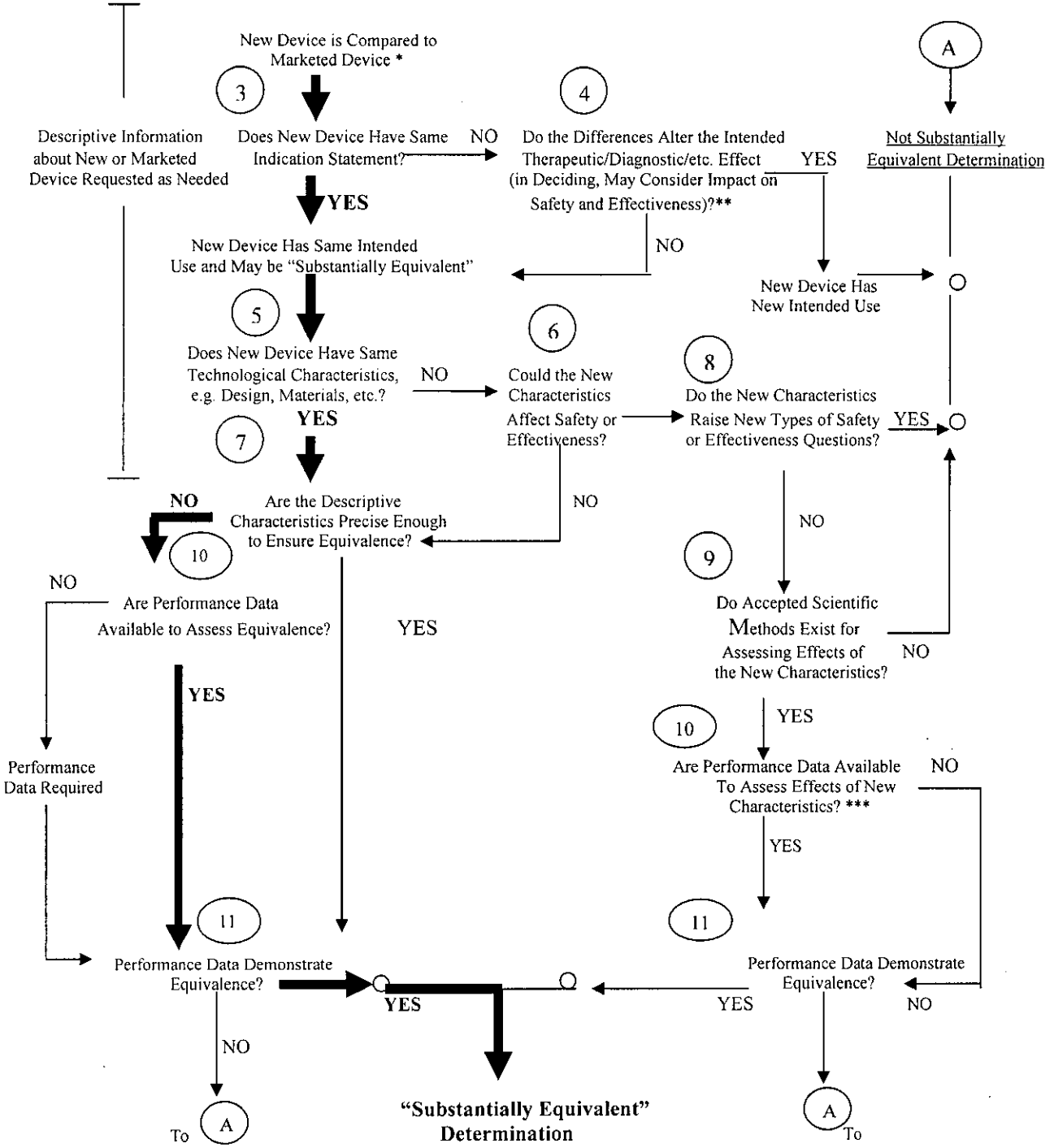
(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

(b)(4) Manufacturing Information

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

510(k) "SUBSTANTIAL EQUIVALENCE" DECISION-MAKING PROCESS



* 510(k) Submissions compare new devices to marketed devices. FDA requests additional information if the relationship between marketed and "predicate" (pre-Amendments or reclassified post-Amendments) devices is unclear.

** This decision is normally based on descriptive information alone, but limited testing information is sometimes required.

*** Data maybe in the 510(k), other 510(k)s, the Center's classification files, or the literature.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

DEPARTMENT OF HEALTH & HUMAN SERVICES **Public Health Service**

U.S. Food and Drug Administration
Center for Devices and Radiological Health
Document Control Center WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

September 09, 2011

ARK DIAGNOSTICS,INC
1190 BORDEAUX DRIVE
SUNNYVALE, CALIFORNIA 94089
ATTN: KENNETH C. KASPER

510k Number: K111904

Product: ARK METHOTREXATE ASSAY, ARK

The additional information you have submitted has been received.

We will notify you when the processing of this submission has been completed or if any additional information is required. Pleaseremember that all correspondence concerning your submission MUST be sent to the Document Mail Center at the above letterhead address. Correspondence sent to any address other than the one above will not be considered as part of your official premarket notification submission. Also, please note the new Blue Book Memorandum regarding Fax and E-mail Policy entitled, "Fax and E-Mail Communication with Industry about Premarket Files Under Review. Please refer to this guidance for information on current fax and e-mail practices at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089402.htm. On August 12, 2005 CDRH issued the Guidance for Industry and FDA Staff: Format for Traditional and Abbreviated 510(k)s. This guidance can be found at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.htm. Please refer to this guidance for assistance on how to format an original submission for a Traditional or Abbreviated 510(k).

The Safe Medical Devices Act of 1990, signed on November 28, states that you may not place this device into commercial distribution until you receive a letter from FDA allowing you to do so. As in the past, we intend to complete our review as quickly as possible. Generally we do so in 90 days. However, the complexity of a submission or a requirement for additional information may occasionally cause the review to extend beyond 90 days. Thus, if you have not received a written decision or been contacted within 90 days of our receipt date you may want to check with FDA to determine the status of your submission.

Please ensure that whether you submit a 510(k) Summary as per 21 CFR 807.92, or a 510(k) Statement as per 21 CFR 807.93, it meets the content and format regulatory requirements.

If you have procedural questions, please contact the Division of Small Manufacturers International and Consumer Assistance (DSMICA) at (301)796-7100 or at their toll-free number (800)638-2041, or contact the 510k staff at (301)796-5640.

Sincerely,

510(k) Staff

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

K111904/S1

K11

ARK Diagnostics, Inc.
Quality Health Through Drug Management

September 7, 2011

Food and Drug Administration
OIVD/CDRH
10903 New Hampshire Avenue
Document Mail Center - WO66-G609
Silver Spring, MD 20993-0002

FDA CDRH DMC
SEP 08 2011
Received

RE: 510(K) Premarket Notification K111904;

ARK™ Methotrexate Assay, ARK™ Methotrexate Calibrator, and ARK™ Methotrexate Control

Dear Reviewing Staff,

(b)(4)

[redacted]

Please feel free to contact me if you have any questions, or require additional information, and please note my contact information below.

Sincerely,

K C Kasper

Kenneth C. Kasper, PhD
Executive Director, Quality and Regulatory Affairs
ARK Diagnostics, Inc.

Tel (408) 747-0708
FAX (408) 747-0783
e-mail: ken@ark-tdm.com

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Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

K111904/S1

September 7, 2011

Kellie B. Kelm, Ph.D.
FDA/CDRH/OIVD/DCTD
Food and Drug Administration
10903 New Hampshire Avenue
WO66, Room 5648
Silver Spring, MD 20993-0002

301-796-6145 - phone
301-847-8513 - fax
kellie.kelm@fda.hhs.gov

FDA CDRH DMC
SEP - 8 2011
Received

RE: 510(K) Premarket Notification K111904, ARK™ Methotrexate Assay
[Note: A written request for an extension was granted. The review period was extended to 01/12/2012 per letter from FDA (M. Shulman, Consumer Safety Officer) dated August 24, 2011.]

Dear Dr. Kelm,

ARK Diagnostics has received your e-mail dated August 17, 2011 regarding the submission "K111904 ARK Methotrexate Assay". You placed the review on telephone hold and requested the following additional information.

Dear Dr. Kasper:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above. We cannot determine if the device is substantially equivalent to a legally marketed predicate device based solely on the information you provided. To complete the review of your submission, we request the following:

(b)(4) Deficiencies

[redacted]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

(b)(4) Deficiencies

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(b)(4) Deficiencies

Records processed under FOIA Request # 2015-8894; Released by CDRH on 02-22-2016

(b)(4) Deficiencies

[redacted]

Please contact me if additional information or clarification is required.

Sincerely,

Kenneth C. Kasper, PhD Executive Director, Quality and Regulatory Affairs
ARK Diagnostics Inc.
1190 Bordeaux Drive Sunnyvale, CA 94089
phone 408-747-0708
fax 408-747-0783

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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ARK™ *Methotrexate Assay*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Assay must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

ARK Diagnostics, Inc.
1190 Bordeaux Drive
Sunnyvale, CA 94089 USA
Tel: 1-877-869-2320
Fax: 1-408-747-0783
customersupport@ark-tdm.com
www.ark-tdm.com

CE EC REP
Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands
Tel: (31) (0) 70 345-8570
Fax: (31) (0) 70 346-7299

Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	R1 R2	Reagent 1/ Reagent 2

© 2011, ARK Diagnostics, Inc.

Reagent Kit REF 5026-0001-00

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Name

ARK™ *Methotrexate Assay*

1 Intended Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

2 Summary and Explanation of the Test

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino]benzoyl]-L-glutamic acid], formerly Amethopterin, is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1-3] Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly. Guidelines for methotrexate therapy with leucovorin rescue should be consulted.[1] Intermediate to high doses of methotrexate (approximately 35 mg/m^2 - 12 g/m^2) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[4-8]

3 Principles of the Procedure

ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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4 Reagents

REF	Product Description	Quantity/Volume
5026-0001-00	**ARK Methotrexate Assay** **Reagent R1 – Antibody/Substrate** rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative, and stabilizers	**1 X 16 mL**
	Reagent R2 – Enzyme methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative, and stabilizers	**1 X 8 mL**

Reagent Handling and Storage

ARK Methotrexate Assay reagents are provided liquid, ready to use and may be used directly from the refrigerator. When not in use, reagents must be stored at 2–8°C (36–46°F), upright and with screw caps tightly closed. If stored as directed, reagents are stable until the expiration date printed on the label. Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C (90°F). **Improper storage of reagents can affect assay performance.**

5 Warnings and Precautions

- For In Vitro Diagnostic Use. For prescription use only.
- Reagents R1 and R2 are provided as a matched set and should not be interchanged with reagents from different lot numbers.

7 Specimen Collection and Preparation for Analysis

- Serum or plasma is required. For consistency, using the same specimen matrix for individual patients is a good practice.
- The sampling time of methotrexate will be dependent on dose, duration of infusion, and clinical status of the patient. Consult specific treatment protocols for sampling time.
- Whole blood cannot be used. The following anticoagulants may be used with this assay.
    - Sodium heparin
    - Lithium heparin
    - Potassium EDTA
- Blood collection must be performed with collection tubes compatible for use with therapeutic drug monitoring (TDM).
- Do not induce foaming and avoid repeated freezing and thawing to preserve the integrity of the specimen from the time it is collected until the time it is assayed.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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- Clarified specimens may be stored up to two weeks at 2 to 8°C. If testing will be delayed, specimens may be stored frozen (≤ -10°C) prior to being tested. Care should be taken to limit the number of freeze-thaw cycles. Specimens were shown to withstand 3 freeze-thaw cycles when stored at -20°C.
- **Handle all patient specimens as if they were potentially infectious.**

8 Procedure

Materials Provided
ARK Methotrexate Assay – REF 5026-0001-00

Materials Required – Provided Separately
ARK Methotrexate Calibrator – REF 5026-0002-00
Quality Controls – ARK Methotrexate Control – REF 5026-0003-00
ARK Methotrexate Dilution Buffer – REF 5026-0004-00

Instruments
Reagents R1 and R2 may need to be transferred to analyzer-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2.

Assay Sequence
To run or calibrate the assay, see the instrument-specific operator's manual.

Calibration
Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E, and F; test calibrators in duplicate. Calibration is required with each new reagent kit lot number. Verify the calibration curve with at least two levels of quality controls according to the established laboratory quality assurance plan.

When to Re-Calibrate
- Whenever a new lot number of reagents is used
- Whenever indicated by quality control results
- Whenever required by standard laboratory protocols

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Quality Control (QC)
Laboratories should establish QC procedures for the ARK Methotrexate Assay. All quality control requirements and testing should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

Good laboratory practice suggests that at least two levels (low and high medical decision points) of quality control be tested each day patient samples are assayed and each time a calibration is performed. Monitor the control values for any trends or shifts. If any trends or shifts are detected, or if the control does not recover within the specified range, review all operating parameters according to your clinical laboratory quality procedures. Contact Customer Service for further assistance.

Manual Dilution Protocol
The range of the ARK Methotrexate calibration curve is 0.00 - 1.20 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.20 µmol/L), are assayed by dilution of the specimen and controls into the range of the calibration curve.

Manually dilute the high specimen or control with ARK Methotrexate Dilution Buffer by preparing the appropriate ten-fold serial dilution as shown below.

	Sample Volume	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000
50 µL	1:1000 sample	450 µL	1:10000	10000

$$\text{Manual Dilution Factor} = \frac{\text{(Volume of Specimen + Volume of Dilution Buffer)}}{\text{Specimen Volume}}$$

Multiply the assayed result by the dilution factor.

9 Results

To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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10 Limitations of Procedure

This assay is designed for use with serum or plasma only; refer to the sections Specimen Collection and Preparation for Analysis. It is generally good practice to use the same method (as well as matrix) consistently for individual patient care due to the potential for method-to-method variability. See the section Expected Values below.

As with all analyte determinations, the methotrexate value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

IMPORTANT: Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay. These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid (DAMPA)[10-12] that result from metabolism of methotrexate by glucarpidase. DAMPA crossreacts with the methotrexate antibody used in this assay, and may continue to circulate for at least five to seven days before accurate measurements of serum methotrexate may return.[13] Oncologists on the clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA that would confuse the efforts of glucarpidase therapy.[13] While glucarpidase is well tolerated and rapidly reduces circulating MTX, delayed renal elimination of MTX can still be a problem for adult and elderly patients.[14]

11 Expected Values

Methotrexate serum levels depend on indication for use, dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors.[1,3] While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example),[15] concentrations may exceed 1000 µmol/L[16] with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.[17] For treatment of osteosarcoma,[16] the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, less than 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin in accordance with algorithms until the MTX level is <0.1 µmol/L. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.[1, 9] Some centers follow ≤ 0.10 µmol/L.[16, 18]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.[1, 9, 19]

Clinical Situation	Laboratory Findings	
	Methotrexate Level (µmol/L)	Hours after administration
Normal Methotrexate Elimination	~10 ~1 <0.2	24 48 72
Delayed Late Methotrexate Elimination	>0.2 >0.05	72 96
Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury	≥50 ≥5 OR ≥100% increase in serum creatinine	24 48 24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs,[14, 19] for example vancomycin.[20] Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.[21-28]

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite,[15, 27] its molar ratio to methotrexate of up to approximately 100-fold,[29] and relative insolubility versus the parent drug,[14, 19] possible nephrotoxicity due to precipitation of the metabolite in renal tubules[29] may delay elimination of methotrexate itself.

Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.[14] Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.[13]

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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12 Specific Performance Characteristics

Each laboratory is responsible for verification of performance using instrument parameters established for their analyzer. The following performance characteristics were obtained on the Roche/Hitachi 917 System.

Limit of Quantitation (LoQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

Measurement Range

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the measurement range.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Recovery

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (monoclonal FPIA)[30].

Methotrexate concentrations by FPIA ranged 0.04 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.04 to 1500 µmol/L. Results of the Passing-Bablok[31] regression analysis for the study are shown below (with 95% confidence limits) for 102 specimens within the measurement range as well as for all 147 specimens including those above the measurement range requiring dilution.

Parameter	Range 0.04 to 1.19 µM		Range 0.04 to 1440 µM	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.00 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.978	(0.968 to 0.985)	0.998	(0.997 to 0.998)
Number of Samples	102	na	147	na

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.1 µmol/L, SD ≤0.01 at ≤0.1 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		Methotrexate (~ 0.50 µmol/L)	
Interfering Substance	**Interferent Concentration**	**Serum Control**	**Test**	**Serum Control**	**Test (% Control)**
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity

Methotrexate's metabolites, folate analogs and other compounds having structural similarity were tested to determine whether these compounds affect the quantitation of methotrexate concentrations using the ARK Methotrexate Assay. High levels of these compounds were spiked into serum pools containing no methotrexate, 0.05 µmol/L or 0.50 µmol/L of methotrexate. The samples were analyzed and the methotrexate concentrations of samples containing interferent were compared to a serum control.

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Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 12 to 48 hours after HDMTX administration.[15, 27, 29, 32, 34-35]

Crossreactivity by 7-hydroxymethotrexate in the measurement of methotrexate was determined for the ARK Methotrexate Assay by testing paired samples containing (1) both 0.05 µmol/L methotrexate and 5 µmol/L 7-hydroxymethotrexate and (2) both 0.50 µmol/L methotrexate and 50 µmol/L 7-hydroxymethotrexate in human serum.

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.

Crossreactivity to 2,4-Diamino-N[10]-methylpteroic acid (DAMPA)

As a minor metabolite of methotrexate, DAMPA is not expected to circulate at concentrations that would interfere in measurement of methotrexate.[33] However, following glucarpidase rescue therapy, the serum concentration of DAMPA can be substantial.[13, 14] The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for MTX cancer treatment due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

		Methotrexate Absent		Methotrexate Present 0.05 µmol/L		Methotrexate Present 0.50 µmol/L	
Compound	**Tested (µmol/L)**	**MTX (µmol/L)**	**Cross Reactivity (%)**	**MTX (µmol/L)**	**Cross Reactivity (%)**	**MTX (µmol/L)**	**Cross Reactivity (%)**
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

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Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

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14 Trademarks

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ARK Diagnostics, Inc.
Sunnyvale, CA 94089 USA

Printed in USA
Revised September 2011
1600-0213-00 Rev 03

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ARK Diagnostics, Inc.

DRAFT

ARK™ *Methotrexate Control*

This ARK Diagnostics, Inc. package insert for the ARK Methotrexate Control must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of the assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

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Key to Symbols Used

LOT	Batch code	YYYY-MM-DD	Use by/Expiration date
REF	Catalog Number		Manufacturer
EC REP	Authorized Representative	CE	CE Mark
IVD	In Vitro Diagnostic Medical Device		Temperature limitation
	Consult Instructions for Use	CONTROL	Quality Control

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Control Kit REF 5026-0003-00

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1 Name

ARK™ *Methotrexate Control*

2 Intended Use

ARK Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay, REF 5026-0001-00.

3 Content

ARK Methotrexate Control is comprised of a synthetic protein matrix with the following concentrations of Methotrexate:

REF	Product Description	Quantity/Volume
5026-0003-00 Complete Set	**ARK Methotrexate Control*** Methotrexate, buffer, bovine serum albumin, and preservatives (nominal level)	Dropper vials
5026-0003-01 Calibration Range Controls[1]	**LOW** (0.07 µmol/L)	1 X 2 mL
	MID (0.40 µmol/L)	1 X 2 mL
	HIGH (0.80 µmol/L)	1 X 2 mL
5026-0003-02 High Range Controls[2]	**5** µmol/L	1 X 2 mL
	50 µmol/L	1 X 2 mL
	500 µmol/L	1 X 2 mL

*To convert results from µmol/L Methotrexate to µg/mL Methotrexate, divide µmol/L by 2.2005. Methotrexate levels become 0.0318, 0.1818, 0.3636, 2.272, 22.72, and 227.2 µg/mL respectively.

The ARK Methotrexate Control is a precision control. Nominal values are not assigned values and are provided only as a guide. Each laboratory should establish the mean value for each control level and its own ranges for each new lot of controls.

[1] Calibration range controls may be obtained separately as a set, REF 5026-0003-01.

[2] High range controls may be obtained separately as a set, REF 5026-0003-02.

4 Warnings and Precautions

- For ***In Vitro*** **Diagnostic** Use.
- Do not mix controls from different lot numbers.
- Use each lot as a set.

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5 Instructions For Use

- For a complete summary and explanation of the Methotrexate assay, refer to the package insert for the ARK Methotrexate Assay.
- Controls are ready to use. Mix each level by gentle inversion before dispensing.
- High range controls must be diluted prior to testing.
- Squeeze sufficient volume (~40µL/drop) into individual sample cups for each level. Consult instrument-specific sample volume requirements. Return caps to their original containers and keep tight.
- Store vials at 2-8°C. Use prior to the expiration date. Once opened, vials may be stored at 2-8°C for 12 months within the expiration date.

6 Limitations of Procedure

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, controls, storage of product as directed, and good laboratory technique.

7 Trademarks

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Sunnyvale, CA 94089 USA

Printed in USA
Revised September 2011
1600-0215-00 Rev 02

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510(k) SUMMARY

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

The assigned 510(k) number is **K111904**.

807.92 (a)(1): Name: **ARK Diagnostics, Inc.**

Address: 1190 Bordeaux Drive
Sunnyvale, CA 94089

Owner Operator Number: 10027663
Establishment Registration: 3005755244

Phone: (408) 747-0700
FAX: (408) 747-0783
Contact: Kenneth C. Kasper, PhD – (408) 747-0708
Executive Director of Quality and Regulatory Affairs

Date prepared: September 7, 2011

807.92 (a)(2): Device name- trade name and common name, and classification

Trade name: ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control

Common Name: Homogeneous Enzyme Immunoassay

Classification: 21 CFR 862 Clinical Chemistry Test System - Toxicology; Test Code LAO; Enzyme Immunoassay, Methotrexate
Pre-Amendment Device, Unclassified
(21 CFR 862.3200 DLJ, 21 CFR 862.3280 LAS)

807.92 (a)(3): Identification of the legally marketed predicate device

Abbott TDx®/TDxFLx® METHOTREXATE II (K932615)

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807.92 (a)(4): Device Description

The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and Methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

The ARK Methotrexate Assay consists of reagents R1 anti-Methotrexate polyclonal antibody with substrate and R2 Methotrexate labeled with bacterial G6PDH enzyme. The ARK Methotrexate Calibrator consists of a six-level set to calibrate the assay, and the ARK Methotrexate Control consists of a six-level set used for quality control of the assay (tri-level calibration range set and tri-level high range set). The ARK Methotrexate Dilution Buffer is equivalent to zero calibrator (Calibrator A).

807.92 (a)(5): Intended Use / Indications for Use

The ARK Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK Methotrexate Calibrator is intended for the calibration of the ARK Methotrexate Assay.

The ARK Methotrexate Control is intended for the quality control of the ARK Methotrexate Assay.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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807.92 (a)(6): Technological Similarities and Differences to the Predicate

SUBSTANTIAL EQUIVALENCE COMPARATIVE CHART

Comparison between the ARK™ Methotrexate Assay and TDx®/TDxFLx® Methotrexate II Assay

Characteristic	Device ARK™ Methotrexate Assay	Predicate Abbott TDx®/TDxFLx® Methotrexate II (K932615)
Intended Use	The ARK™ Methotrexate Assay is intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers.	The TDx®/TDxFLx® Methotrexate II assay is a reagent system for the quantitative measurement of methotrexate, an antineoplastic drug, in serum or plasma.
Indications for Use	The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.	The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.
Sample	Serum or plasma	Serum or plasma
Methodology	Homogenous enzyme immunoassay (EIA)	Fluorescence polarization immunoassay (FPIA)
Reagent Components	Two (2) reagent system: Anti-Methotrexate Antibody/Substrate Reagent (R1) containing rabbit polyclonal antibodies to Methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservatives, and stabilizers Enzyme Reagent (R2) containing Methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservatives, and stabilizers	Reagent Pack: W = Wash solution, solvent, sodium azide S = Methotrexate Antibody (mouse monoclonal), buffer, protein and sodium azide T = Methotrexate Fluorescein Tracer, buffer, protein, surfactant and sodium azide P = Pretreatment Solution, protein, surfactant and sodium azide
Platform required	Automated clinical chemistry analyzer	TDx clinical chemistry analyzer
Accessory reagents	Calibrators (six levels) and controls (six levels) in a synthetic matrix; Dilution Buffer	Calibrators (six levels) and controls (six levels) in human serum; Dilution Buffer
Testing environment	Routine clinical laboratory	Routine clinical laboratory
Reagent condition and storage	Liquid, 2-8° C	Liquid, 2-8° C

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807.92 (b)(1) and 807.92 (b)(2):
Brief Description of Nonclinical and Clinical Data

Limit of Quantitation (LOQ)

The following characteristics were determined according to CLSI EP17-A for the ARK Methotrexate Assay. Analyzer-specific performance may vary.

Criterion	MTX Concentration (µmol/L)
Limit of Blank (LoB); N = 60 µB + 1.645 SD , where SD = 0.005	0.01
Limit of Detection (LoD); N = 60 LoB + 1.652 SD, where SD = 0.005	0.02
Limit of Quantitation (LoQ); N = 40 LoQ – 2 SD > LoD	0.04

Each laboratory is responsible for determining reporting criteria for methotrexate concentrations. The following suggestion from CLSI EP17-A may be appropriate:

Result ≤ LoB	report "not detected; concentration < LoD"
LoB < Result < LoQ	report "analyte detected; concentration < LoQ"
Result ≥ LoQ	report the result as measured

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Accuracy

Accuracy (analytical recovery) was performed by adding concentrated methotrexate drug into human serum negative for methotrexate. A certified stock concentrate of highly pure methotrexate was added volumetrically to human serum negative for methotrexate, representing drug concentrations across the assay calibration range. Six replicates of each sample were assayed on an automated clinical chemistry analyzer. The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = 100 \times \frac{\text{Mean recovered concentration}}{\text{Target concentration}}$$

Theoretical Concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percentage Recovery
0.06	0.07	111.1
0.10	0.10	100.0
0.30	0.30	98.3
0.60	0.61	102.2
1.00	0.99	98.8

Mean percentage recovery: 102.1

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Linearity

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. A 1.30 µmol/L serum sample was prepared and dilutions were made proportionally with human serum negative for methotrexate. Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values for concentrations >0.10 µmol/L or ±0.01 µmol/L at concentrations ≤ 0.10 µmol/L. Results are shown below.

Theoretical (µmol/L)	Observed Results (µmol/L)	1st Order Predicted Results	2nd Order Predicted Results	Difference (µmol/L or %)
0.00	0.00	0.009	-0.003	na
0.02	0.02	0.026	0.016	-0.010 µmol/L
0.04	0.04	0.042	0.034	-0.008 µmol/L
0.05	0.06	0.059	0.053	-0.006 µmol/L
0.07	0.08	0.076	0.072	-0.004 µmol/L
0.11	0.11	0.110	0.109	-0.7 %
0.18	0.17	0.178	0.183	3.1 %
0.36	0.34	0.347	0.364	4.8 %
0.65	0.63	0.618	0.639	3.4 %
0.72	0.72	0.686	0.705	2.9 %
0.86	0.84	0.821	0.835	1.7 %
1.01	0.99	0.957	0.960	0.4 %
1.15	1.06	1.092	1.082	-1.0 %
1.30*	1.19	1.228	1.199	-2.3 %

*Concentration exceeds the claimed calibration range.

Samples containing methotrexate between 2 and 1200 µmol/L were prepared proportionally in pooled human serum and then diluted into the calibration range with ARK Methotrexate Dilution Buffer. Regression of assayed methotrexate concentrations was linear throughout the range.

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Assay Range

The measurement range of the ARK Methotrexate Assay is 0.04 - 1.20 µmol/L. Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen. Report assayed values exceeding the LoD according to the information provided for LoQ. Multiply the assayed result by the dilution factor for specimens containing methotrexate above the measurement range.

Method Comparison

Correlation studies were performed using CLSI/NCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (monoclonal FPIA).

Methotrexate concentrations by FPIA ranged 0.04 to 1440 µmol/L (µM). ARK Methotrexate values ranged 0.04 to 1500 µmol/L. Results of the Passing-Bablok regression analysis for the study are shown below (with 95% confidence limits) for 102 specimens within the measurement range as well as for all 147 specimens including those above the measurement range requiring dilution.

Parameter	**Range 0.04 to 1.19 µM**		**Range 0.04 to 1440 µM**	
Slope	1.00	(1.00 to 1.02)	0.99	(0.96 to 1.00)
y-intercept	0.01	(0.00 to 0.01)	0.01	(0.01 to 0.01)
Correlation Coefficient (r^2)	0.978	(0.968 to 0.985)	0.998	(0.997 to 0.998)
Number of Samples	102	na	147	na

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Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. The six-level ARK Methotrexate Control and pooled human specimens containing methotrexate were used in the study. Each level was assayed in quadruplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within-run, between-day, total SD, and percent CVs were calculated. Results are shown below. Acceptance criteria: ≤10% total CV at >0.10 µmol/L, SD ≤0.01 at ≤0.10 µmol/L.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	%CV	SD	%CV	SD	%CV
ARK Methotrexate Control								
LOW	160	0.06	0.005	8.1	0.005	7.1	0.007	10.6
MID	160	0.37	0.011	3.1	0.008	2.2	0.014	3.8
HIGH	160	0.76	0.039	5.1	0.029	3.8	0.048	6.4
5	160	4.8	0.13	2.8	0.013	2.8	0.19	4.1
50	160	48	1.40	2.9	2.13	4.4	2.71	5.6
500	160	470	15.63	3.3	27.64	5.8	33.35	7.0
Patient Pool								
LOW	160	0.07	0.006	9.1	0.005	7.5	0.008	11.7
MID	160	0.41	0.013	3.3	0.026	6.4	0.030	7.2
HIGH	160	0.82	0.037	4.5	0.042	5.1	0.057	6.9
5	160	4.6	0.14	3.1	0.18	4.0	0.24	5.3
50	160	45	1.31	2.9	2.62	5.9	2.92	6.5
500	160	460	11.55	2.5	27.21	5.9	29.63	6.4

Questions? Contact FDA/CDRH/OCE/DID at CDRH-FOISTATUS@fda.hhs.gov or 301-796-8118

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Interfering Substances

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of methotrexate was not substantially affected at the levels of endogenous substances tested.

		Methotrexate (~ 0.05 µmol/L)		Methotrexate (~ 0.50 µmol/L)	
Interfering Substance	**Interferent Concentration**	**Serum Control**	**Test**	**Serum Control**	**Test (% Control)**
Albumin	12 g/dL	0.05	0.06	0.48	0.45 (92.8)
Bilirubin - conjugated	70 mg/dL	0.05	0.06	0.48	0.51 (105.5)
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	0.48	0.52 (106.9)
Cholesterol	400 mg/dL	0.05	0.06	0.47	0.49 (105.4)
Gamma-Globulin	12 g/dL	0.05	0.06	0.48	0.51 (105.5)
Hemoglobin	1000 mg/dL	0.04	0.05	0.49	0.45 (92.8)
Intralipid®	500 mg/dL	0.05	0.05	0.43	0.45 (105.1)
Rheumatoid Factor	1100 IU/mL	0.05	0.06	0.43	0.41 (96.1)
Triglycerides	749 mg/dL	0.04	0.04	0.49	0.45 (91.4)
Uric Acid	30 mg/dL	0.05	0.04	0.48	0.50 (102.8)

Specificity - Drug Interference

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate Assay did not crossreact (≤ 0.07%) with the major metabolite 7-Hydroxymethotrexate.

Crossreactivity to the minor, inactive metabolite 2,4-diamino-N^{10}-methylpteroic acid (DAMPA)

The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA. Tests were performed in the absence of the parent drug methotrexate. Crossreactivity to DAMPA ranged 64.3 to 100%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Drugs that crossreact

The ARK Methotrexate Assay crossreacts slightly with triamterene and trimethoprim, however these drugs may be contraindicated for methotrexate cancer treatement due to additional adverse effects if co-administered. The structures of these compounds closely match the pteridine ring moiety of methotrexate.

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Compound	Tested (µmol/L)	Methotrexate Absent MTX (µmol/L)	Methotrexate Absent Cross Reactivity (%)	Methotrexate Present 0.05 µmol/L MTX (µmol/L)	Methotrexate Present 0.05 µmol/L Cross Reactivity (%)	Methotrexate Present 0.50 µmol/L MTX (µmol/L)	Methotrexate Present 0.50 µmol/L Cross Reactivity (%)
Triamterene	25	0.46	1.85	0.89	3.32	1.04	2.31
Trimethoprim	100	0.17	0.17	0.16	0.12	0.99	0.54

Crossreactivity to folate analogs and other compounds

The ARK Methotrexate Assay did not crossreact (≤ 0.01%) with folate analogs or other compounds at ≥ 1000 µmol/L as tested.

Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000
Sulfamethoxazole	1600
Tetrahydrofolic Acid	1000
Vinblastine	1000
Vincristine	1000

Anticoagulants

Studies were conducted to determine the performance characteristics of the assay for both serum and plasma samples containing methotrexate.

The results indicate that there is no significant difference between the recovery of methotrexate in serum or plasma.

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Sample Stability

Human specimens were shown to be stable frozen (at least 15 months), forty-eight (48) hours at room temperature, refrigerated (at least 21 days) and after three (3) successive freeze/thaw cycles.

On-Board Stability

Calibration Curve Stability: A stored calibration was effective up to at least 19 days based on supporting data.

Reagent on-board stability: Reagents were effective when stored after transfer to analyzer specific reagent containers for up to at least 25 days based on supporting data. In-use stability of calibrator and controls was also demonstrated.

807.92 (b)(3): Conclusions from Nonclinical Testing

As summarized above, the ARK Methotrexate Assay System, including the ARK Methotrexate Calibrator, ARK Methotrexate Control and ARK Methotrexate Dilution Buffer, is substantially equivalent to the Abbott TDx®/TDxFLx® METHOTREXATE II Assay system. The ARK Methotrexate Assay system was shown to be safe and effective for its intended use based on performance studies.

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Indication for Use

510(K) Number (if known): K111904

Device Name:

ARK™ Methotrexate Assay
ARK™ Methotrexate Calibrator
ARK™ Methotrexate Control

Indications for Use:

The ARK™ Methotrexate Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of Methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

The ARK™ Methotrexate Calibrator is intended for use in calibration of the ARK Methotrexate Assay.

The ARK™ Methotrexate Control is intended for use in quality control of the ARK Methotrexate Assay.

Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should ***not*** be tested with the ARK Methotrexate Assay.

Prescription Use ___X___	And/Or	Over the Counter Use ____.
(21 CFR Part 801 Subpart D)		(21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) 111904

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