Roche Diagnostics Operations
Elina Voronovsky
Regulatory Affairs Manager
9115 Hague Road
Indianapolis, Indiana 46250

Re: K233454
Trade/Device Name: ONLINE TDM Methotrexate
Regulatory Class: Unclassified
Product Code: LAO
Dated: January 12, 2024
Received: January 12, 2024

Dear Elina Voronovsky:

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 (the 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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Joseph A. Kotarek -S
Digitally signed by Joseph A. Kotarek -S
Date: 2024.02.20 21:55:40 -05'00'

Joseph Kotarek
Branch Chief
Division of Chemistry
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Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

Submission Number *(if known)*

K233454

Device Name

ONLINE TDM Methotrexate

Indications for Use *(Describe)*

ONLINE TDM Methotrexate is an in vitro test for the quantitative determination of methotrexate in human serum and plasma on cobas c systems. The determination of methotrexate is used for monitoring levels of methotrexate to ensure appropriate therapy.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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ONLINE TDM Methotrexate
K233454 – 510(k) Summary

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

Submitter Name	Roche Diagnostics
Address	9115 Hague Road P.O. Box 50416 Indianapolis, IN 46250-0457
Contact	Elina Voronovsky Phone: (317) 478-3317 Email: elina.voronovsky.ev1@roche.com
Date Prepared	February 20, 2024
Proprietary Name	ONLINE TDM Methotrexate
Common Name	ONLINE TDM Methotrexate
Classification Name	Enzyme Immunoassay, Methotrexate
Product Codes, Regulation Numbers	LAO, Unclassified
Predicate Devices	ARK Methotrexate Assay (k111904)
Establishment Registration	Roche Diagnostics GmbH Mannheim, Germany: 9610126 Roche Diagnostics GmbH Penzberg, Germany: 9610529 Roche Diagnostics Indianapolis, IN United States: 1823260

1. DEVICE DESCRIPTION

The ONLINE TDM Methotrexate assay is an in vitro test for the quantitative determination of methotrexate in human serum and plasma on **cobas c** systems. The determination of methotrexate is used for monitoring levels of methotrexate to ensure appropriate therapy.

The ONLINE TDM MTX assay is a two-reagent system used for the detection of methotrexate in serum and plasma. In this technology drug hapten attached to the enzyme glucose 6 phosphate dehydrogenase (G6PDH) serves as the binding partner to anti-methotrexate antibody. A competitive reaction to a limited amount of specific anti-methotrexate antibody takes place between the enzyme bound hapten and free methotrexate in the sample. Enzyme activity is reduced with bound antibody. Only active enzymes reduce NAD+ to NADH. The rate of NADH formation during the reaction correlates to the methotrexate concentration and is measured photometrically.

The ONLINE TDM MTX assay is a homogeneous enzyme-immunoassay.

Reagents - working solutions

R1: Anti-methotrexate antibody (rabbit monoclonal), 3 µg/mL; NAD, G6P, bovine serum albumin in water, pH 6.3; preservative

R3: Methotrexate hapten conjugated to G6PDH, 0.3 µg/mL; bovine serum albumin in buffer, pH 7.8; preservative

2. INDICATIONS FOR USE

ONLINE TDM Methotrexate is an in vitro test for the quantitative determination of methotrexate in human serum and plasma on **cobas c** systems. The determination of methotrexate is used for monitoring levels of methotrexate to ensure appropriate therapy.

3. TECHNOLOGICAL CHARACTERISTICS

The following table compares the ONLINE TDM Methotrexate assay on **cobas c** 503 with its predicate device, ARK Methotrexate Assay (k111904).

Table 1: ONLINE TDM Methotrexate Technical Characteristics

	Candidate Device: ONLINE TDM Methotrexate Assay	**Predicate Device: ARK Methotrexate Assay (k111904)**
Intended Use / Indications for Use	The Roche ONLINE TDM Methotrexate Assay is intended as the In vitro test for the quantitative determination of methotrexate in human serum and plasma on **cobas c** systems. The determination of methotrexate is used for monitoring levels of methotrexate to ensure appropriate therapy.	The ARK™ Methotrexate Assay is intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers.
Assay Method	Same	Homogenous enzyme immunoassay (EIA)
Detection Method	Same	Absorbance
Instrument Platform	Same	Automated clinical chemistry analyzer
Sample Type/Matrix	Same	Serum or plasma
Calibrator	Roche MTX Calibrator	ARK Methotrexate Calibrator
Calibration Method	1 calibrator diluted to 6 levels by the instrument. The calibrator consists of a synthetic protein matrix and 1.2 µmol/L.	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
Calibration Interval	- after reagent lot change - every 2 weeks - as required following quality control procedures	- Whenever a new lot number of reagents is used - Whenever indicated by quality control results - Whenever required by standard laboratory protocols
Controls	Roche TDM Control	ARK Methotrexate Control

	Candidate Device: ONLINE TDM Methotrexate Assay	Predicate Device: ARK Methotrexate Assay (k111904)
Traceability/ Standardization	Roche MTX Calibrator is prepared to contain known quantities of methotrexate in a synthetic proteinaceous matrix free of methotrexate and is traceable to USP reference standard.	ARK Methotrexate Calibrators and controls are prepared by volumetric dilution of high purity, certified Methotrexate solution into a synthetic proteinaceous matrix free of Methotrexate
Reagent Stability	Shelf life at 2-8 °C: See expiration date on **cobas c** pack label On-board in use and refrigerated on the analyzer: 12 weeks	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.
Measuring Range	Same	0.04 – 1.20 µmol/L Specimens containing methotrexate in higher concentrations are assayed by dilution of the specimen.
Lower Limits of Measurement	LoB = 0.0250 µmol/L LoD = 0.0350 µmol/L LoQ = 0.0400 µmol/L	LoB = 0.01 µmol/L LoD = 0.02 µmol/L LoQ = 0.04 µmol/L

4. NON-CLINICAL PERFORMANCE EVALUATION

Performance characteristics were evaluated with ONLINE TDM Methotrexate on **cobas c** 503 and are briefly summarized below.

All acceptance criteria were met.

4.1. Precision

4.1.1. Repeatability and Intermediate Precision

Precision was determined in accordance with the CLSI EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer. The results are summarized below. All acceptance criteria were met.

Repeatability	Mean	SD	CV
	µmol/L	µmol/L	%
Control 1	0.0863	0.00376	4.4
Control 2	0.485	0.00421	0.9
Control 3	0.849	0.00567	0.7
Human Serum 1	0.0872	0.00350	4.0
Human Serum 2	0.526	0.00427	0.8
Human Serum 3	0.889	0.00648	0.7
Human Serum 4	4.85	0.0491	1.0
Human Serum 5	44.2	1.76	4.0
Human Serum 6	449	16.6	3.7
Human Serum 7	1334	41.8	3.1

Intermediate precision	Mean	SD	CV
	µmol/L	µmol/L	%
Control 1	0.0737	0.00804	10.9
Control 2	0.487	0.00561	1.2
Control 3	0.841	0.00664	0.8
Human Serum 1	0.0752	0.00845	11.2
Human Serum 2	0.526	0.00683	1.3
Human Serum 3	0.889	0.00971	1.1
Human Serum 4	4.91	0.0970	2.0
Human Serum 5	44.2	2.36	5.3
Human Serum 6	449	28.5	6.3
Human Serum 7	1316	67.3	5.1

4.2. Analytical Sensitivity

4.2.1. Limit of Blank (LoB)

For determination of LoB, one analyte-free sample was measured with three reagent lots in 6 runs, each run with 10-fold determination, distributed over 3 days, on one **cobas c** 503 analyzer. The LoB was determined according to CLSI EP17-A2. The LoB claim in the labeling will be set to ≤ 0.0250 µmol/L.

4.2.2. Limit of Detection (LoD)

For determination of LoD, 5 serum samples (spiked with methotrexate) were measured on three lots with 2-fold determination per run on one **cobas c** 503 analyzer. Six runs were distributed over 3 days. The LoD was determined according to CLSI EP17-A2. The LoD claim in the labeling will be set to ≤ 0.0350 µmol/L.

4.2.3. Limit of Quantitation (LoQ)

For determination of LoQ, 5 serum samples (spiked with methotrexate) were measured with three reagent lots on one **cobas c** 503. Six runs were distributed over 3 days. The Limit of Quantitation (LoQ) was determined according to CLSI EP17-A2. The LoQ claim in the labeling will be set to 0.0400 µmol/L.

4.3. Linearity/Assay Reportable Range

The linearity of the ONLINE TDM Methotrexate assay was assessed according to CLSI EP06-A-Ed2.

A dilution series was prepared from human serum spiked with methotrexate (sample High) and analyte-free human serum (sample Blank). The dilution series spanning the measuring range was prepared to obtain ≥ 9 levels. Samples were assayed on one **cobas c** 503 analyzer using 3 reagent lots and 4 replicates per sample. The linearity data was analyzed according to CLSI EP06-Ed2.

Linearity was confirmed for the measuring range of 0.0400 – 1.20 µmol/L.

4.4. Dilution

Post Dilution Check experiments were performed for samples above the measuring range. Human serum samples were spiked with methotrexate and measured with the ONLINE TDM Methotrexate assay on the **cobas c** 503 via instrument and/or manual dilution, depending on the concentration level. The target value was determined with the LC-MS/MS.

4.5. Endogenous Interferences

Endogenous substances (conjugated and unconjugated bilirubin, hemoglobin, lipemia (Intralipid), Immunoglobulin G (IgG), albumin, rheumatoid factor, total protein,) were evaluated for potential interference with the ONLINE TDM Methotrexate assay on the **cobas c** 503 analyzer.

All predefined acceptance criteria were met, and the proposed labeling claims for each endogenous substance can be found below:

Endogenous Substance	**Claim** **No interference up to**
Icterus	I index of 60 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).
Hemolysis	H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL)
Lipemia (Intralipid)	L index of 1000
Albumin	60 g/L
Immunoglobulin G	60 g/L
Total protein	between concentrations of 2-12 g/dL
Rheumatoid factors	1000 IU/mL

4.6. Analytical Specificity/Cross-Reactivity

DAMPA (4-[(2,4-Diaminopteridin-6-yl) methyl-methylamino] benzoic acid was tested for cross-reactivity with ONLINE TDM Methotrexate on the **cobas c** 503 analyzer. Two human serum sample pools were spiked with methotrexate to approximately 0.1 and 1.0 µmol/L and then divided into 2 portions. One portion of each pool was spiked with 0.1 µmol/L of DAMPA and the other portion of the pool with solvent used to dissolve DAMPA, which was used for the baseline reference methotrexate concentration. The methotrexate concentration was determined at least in 5-fold determination and compared to the reference aliquot.

DAMPA cross-reactivity was shown at 87.6 % at methotrexate level 0.1 µmol/L and at 64.6 % at methotrexate level 1.0 µmol/L.

DAMPA can cross-react between 50 % and 150 %.

4.7. Exogenous Interferences – Drugs

An exogenous interference study was conducted to evaluate commonly used pharmaceuticals and in addition, special pharmaceuticals were tested with ONLINE TDM Methotrexate on the **cobas c** 503 analyzer. All acceptance criteria were met.

Drug	Concentration tested (mg/L)
Acetaminophen	156
N-Acetylcysteine	150
Acetylsalicylic acid	30
Ampicillin-Na	75
Ascorbic acid	52.5
Cefoxitin	750
Cyclosporine	1.8
Doxycyclin	18
Heparin	3300 IU/L
Ibuprofen	219
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Phenylbutazone	321
Rifampicin	48
Theophylline	60
5-Fluorouracil	90
5-Methyl-THF	459
6-Mercaptopurin	1.48
6-Methyl-5,6,7,8-tetrahydropterin-dihydrochlorid	254
7-Hydroxy methotrexate	9.35
Adriamycin	580
Carbamazepine	45
Chloramphenicol	78
Cisplatin	15
Cyclophosphamide	549
Cytosine	111
Digoxin	0.039
Dihydrofolic acid	443
Disopyramide	16.8

Erythromycin	138
Folic acid	0.44
Furosemide	15.9
Gabapentin	26.7
Hydrochlorothiazide	1.13
Isoproterenol hydrochloride	0.0595
Leucovorin	1420
Lidocaine	15
Naproxen	360
Phenobarbital	690
Phenytoin	60.0
Prednisolone	1.2
Prednisone	0.099
Pyrimethamine	249
Sulfamethoxazole	405
Tetrahydrofolic acid	445
Triamterene	0.585
Trimethoprim	42.0
Vancomycin	120
Vinblastine	811
Vincristine	825

4.8. Sample Matrix Comparison

The effect on quantitation of methotrexate values in the presence of anticoagulants with the ONLINE TDM Methotrexate assay was determined on the **cobas c** 503 analyzer by comparing values obtained from samples collected in serum, Li-Heparin, K2-EDTA, K3-EDTA, and Na-Heparin plasma tubes. The study was performed using ≥ 50 samples, 1 lot of reagent and measured on 1 **cobas c** 503 analyzer. All predefined acceptance criteria were met, supporting the labeling claim that serum, Li-Heparin, K2-EDTA, K3-EDTA, and Na-Heparin are acceptable sample types.

Anticoagulant	Slope	Intercept (µmol/L)	Correlation Coefficient (Pearson r)	Concentration of Samples (µmol/L)
Serum vs. Li-Heparin plasma	1.005	-0.00539	0.998	0.0642 – 1.19

Serum vs. K2-EDTA plasma	0.997	0.00309	0.999	0.0421 – 1.19
Serum vs. K3-EDTA plasma	1.003	-0.00261	0.998	0.0642 – 1.19
Serum vs. Na-Heparin plasma	1.000	-0.00300	0.998	0.0642 – 1.19

4.9. Method Comparison to Validated Comparator Method

A method comparison of the ONLINE TDM Methotrexate on the **cobas c** 503 analyzer versus a validated comparator method on LC-MS/MS was completed. 105, native human plasma and serum samples, were tested on a **cobas c** 503 analyzer and LC-MS/MS, in singlet using 1 lot of reagent. The sample concentrations were between 0.0429 µmol/L and 1.19 µmol/L. The results can be found below:

Deming Regression

$y = 1.032x – 0.000831$ µmol/L

$r = 0.997$

4.10. Stability

Stability studies were conducted to support Roche Diagnostic's claims as reported in the package labeling.

5. CONCLUSIONS

The analytical performance data for ONLINE TDM Methotrexate assay met the acceptance criteria and support the substantial equivalence of ONLINE TDM Methotrexate assay on **cobas c** 503 analyzer to ARK Methotrexate Assay.