510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232017

B Applicant

ARK Diagnostics, Inc.

C Proprietary and Established Names

ARK Methotrexate II Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LAO	Unclassified		

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Methotrexate

C Type of Test:

Quantitative Homogenous Enzyme Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

B Indication(s) for Use:

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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

All performance studies were conducted on the Beckman Coulter AU680 Clinical Chemistry Analyzer.

IV Device/System Characteristics:

A Device Description:

The ARK Methotrexate II Assay consists of reagents R1 (anti-Methotrexate rabbit monoclonal antibody with substrate) and R2 (Methotrexate labeled with bacterial G6PDH enzyme).

B Principle of Operation:

The ARK Methotrexate II 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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ARK Methotrexate Assay

B Predicate 510(k) Number(s):
K111904

C Comparison with Predicate(s):

Device & Predicate Device(s):	K232017	K111904
Device Trade Name	ARK Methotrexate II Assay	ARK Methotrexate Assay

General Device Characteristic Similarities		
Intended Use/Indications For Use	The ARK Methotrexate Assay is intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers.	Same
Sample Matrix	Human serum or plasma	Same
Analyte	Methotrexate	Same
User Environment	Professional Clinical Laboratory: Prescription Use Only	Same
General Device Characteristic Differences		
Measurement range	0.03 – 1.30 µmol/L	0.04 – 1.20 µmol/L

VI Standards/Guidance Documents Referenced:

- CLSI document EP05-A3, *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline -Third Edition*
- CLSI Guideline EP06-A2: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach -Second Edition*
- CLSI Protocol EP37; *Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition*
- CLSI Protocol EP17-A2 *Evaluation of Detection Capability for Clinical Laboratory*
- *Measurement Procedures: Approved Guideline – Second Edition*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Performance was validated on the Beckman Coulter AU680 instrument.

1. Precision/Reproducibility:

A precision study was performed in accordance with CLSI EP05-A3. Three levels of controls and three corresponding pooled human serum methotrexate samples were used in the study. The methotrexate concentration in Low, Mid, and High human serum pools were spiked to approximate the same levels as the controls. Each level of control and patient sample was

assayed in quadruplicate twice a day over twenty days for a total of 160 determinations per sample per lot.

Mean methotrexate concentration, standard deviation (SD) and coefficients of variations (%CVs) were calculated for within-run (repeatability), between-day, and total precision.

Total precision results are summarized below:

		Within Run		Between Day		Total	
Sample	**Mean (µmol/ L)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Controls							
LOW	0.069	0.002	2.84	0.001	1.23	0.002	3.00
MID	0.411	0.006	1.40	0.002	0.43	0.006	1.40
HIGH	0.811	0.014	1.79	0.008	0.97	0.017	2.05
Patient Pools							
LOW	0.070	0.002	2.50	0.001	1.49	0.002	2.88
MID	0.404	0.008	1.86	0.003	0.65	0.008	1.92
HIGH	0.846	0.016	1.93	0.008	0.95	0.017	2.06

The sponsor also performed a 5-day precision study to evaluate serum and plasma samples. The results showed that precision for serum and plasma was not significantly different.

2. Linearity/assay reportable range:

Linearity study was performed by spiking human serum negative for methotrexate. Twelve (12) dilutions were created by mixing the high human serum pool with the low human serum pool. Three (3) replicates were measured for each panel member using a single reagent lot on one Beckman Coulter AU680 clinical chemistry analyzer. The data were analyzed using a weighted linear regression model. The deviation from linearity did not exceed 8.76%.

Linear regression analysis yields the following result:
$y = 1.0886x - 0.0004$
$R^2 = 0.9984$

The claimed measuring range of this device is 0.030 - 1.300 µmol/L.

Evaluation of recovery:

An accuracy-by-recovery study was conducted to determine the trueness of quantitative measurements of methotrexate across the measurement range of the ARK Methotrexate II Assay. Samples were prepared by volumetric addition of methotrexate to human serum negative for methotrexate. Drug concentrations across the assay range were tested. Each sample was assayed in triplicate in each of two separate analytical runs for a total of six

replicates. The results were averaged and compared to the theoretical target concentration and percentage recovery calculated.

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

Target concentration (µmol/L)	Mean Recovered Concentration (µmol/L)	Percent Recovery (%)
0.060	0.063	104.4
0.100	0.105	105.2
0.300	0.322	107.2
0.600	0.628	104.7
1.000	1.079	107.9
1.200	1.293	107.8

Evaluation of linearity for samples with elevated methotrexate levels:

A sample containing approximately 1200 µmol/L of methotrexate 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 II Dilution Buffer. Ten-fold dilution factors were used. Two separate analytical runs of three replicates of each sample per run (N=6).

The results showed that the recovery ranged from 91.6% to 105.6% for all concentration tested.

Expected (µmol/L)	2	5	8	20	50	80	200	500	800	1200
Dilution	1/10	1/10	1/10	1/100	1/100	1/100	1/1000	1/1000	1/0000	1/1000
Mean (µmol/L)	2.1	4.6	8.4	20.1	46.4	83.0	198.5	457.8	825.3	1199
SD	0.03	0.09	0.12	0.41	0.52	1.15	1.87	9.20	13.87	20.27
CV (%)	1.7	1.9	1.4	2.1	1.1	1.4	0.9	2.0	1.7	1.7
N	6	6	6	6	6	6	6	6	6	6
Recovery (%)	103.2	92.7	105.6	100.3	92.8	103.8	99.3	91.6	103.2	99.9

Evaluation of accuracy in samples with elevated methotrexate levels:

A method comparison study was performed to evaluate the agreement between the ARK Methotrexate II Assay and the predicate device ARK Methotrexate Assay using samples with elevated methotrexate levels (i.e., samples that require dilution into the assay's measuring range for evaluation).

Forty-two (42) native specimens (neat) with methotrexate levels exceeding the measurement range, five (5) samples contrived by volumetric addition methotrexate drug into existing patient specimens, and four (4) samples contrived by pooling patient specimens were tested (for a total of 51 samples). The samples ranged from 1.430 – 1178 µmol/L.
Results of the study are summarized in the table below:

Parameters	**Range from 1.43 to 1178 µmol/L**
Number of Samples	51
Slope	0.95 (0.93 to 0.98)
Y-intercepts	0.09 (-0.25 to 0.52)
Correlation Coefficient (r^2)	0.99 (.99 to 1.00)

Evaluation of precision in samples with elevated methotrexate levels:
Precision was evaluated as described above in 1.
ARK Methotrexate High Range Controls are comprised of three levels (5, 50, and 500 µmol/L).

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 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) were spiked with methotrexate.

		Within Run		**Between Day**		**Total**	
Sample	**Mean (µmol/L)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Controls							
5	4.868	0.070	1.44	0.036	0.74	0.077	1.58
50	49.660	1.108	2.23	0.397	0.80	1.141	2.30
500	493.769	8.012	1.62	2.483	0.50	8.012	1.62
Patient Pools							
5	5.247	0.076	1.45	0.028	0.54	0.078	1.49
50	51.614	0.723	1.40	0.285	0.55	0.777	1.51
500	507.988	7.632	1.50	4.240	0.83	8.538	1.68

3. Analytical Specificity/Interference:

Potential interference of endogenous materials were evaluated based upon recommendations in the Clinical and Laboratory Standard Institute (CLSI) guideline EP-07 3rd edition. Endogenous serum samples with low (0.050 µmol/L) or high (0.500 µmol/L) levels of methotrexate were spiked with potentially interfering substances. Percent interference was calculated using the following equation:

$$100 \times \frac{\text{(mean result of the test substances)} - \text{(mean result of the control samples)}}{\text{mean result of the control sample}}$$

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.

The interference study results are summarized below:

Endogenous Substance	**Highest Concentration tested without significant interference**
Albumin	12 g/dL
Bilirubin- conjugated	72 mg/dL
Bilirubin- unconjugated	72 mg/dL
Cholesterol	500 mg/dL
Human IgG	12 g/dL
Hemoglobin	1000 mg/dL
Rheumatoid Factor	1080 IU/mL
Triglycerides	1000 mg/dL
Uric Acid	30 mg/dL

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™ II 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) and 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 were added to serum either in the absence of methotrexate or to serum containing methotrexate.

Metabolites

Cross reactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate II Assay was not substantially affected by the presence of its major metabolite when tested at 50 µmol/L 7-OH-MTX.

Metabolite	7-OH-MTX µmol/L	Interference (%)	
		Methotrexate 0.050 µmol/L	Methotrexate 0.500 µmol/L
7-Hydroxymethotrexate	50	8.72%	0.58%

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

The ARK Methotrexate II 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 from 18.80% to 57.50%. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

Other Compounds

The ARK II Methotrexate Assay resulted in ≤10% interference in the presence of drug compounds at the levels tested:

Compound	Highest Concentration tested without significant interference (µmol/L)
Adriamycin	1000
Cyclophosphamide	2200
Cytosine	1000
Dihydrofolic Acid	1000
Tetrahydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic Acid	1000
Prednisolone	1000

Pyrimethamine	1000
Sulfamethoxazole	1600
Vinblastine	1000
Vincristine	1000
Trimethoprim	150
Triamterene	25

4. Assay Reportable Range:
   See linearity section (1 b.) above.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   ARK Methotrexate II Assay is traceable to commercially available material.

6. Detection Limit:

   Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ) studies were conducted using the CLSI Guideline EP 17-2A. These studies were performed on the Beckman Coulter AU680 clinical chemistry analyzer.

   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. LoB was calculated using the non-parametric method.

   LoQ of 0.030 µmol/L was determined using four levels of pooled human serum supplemented with methotrexate concentrations ranging from 0.02 to 0.05 µmol/L. Eight (8) replicates of each LoQ sample were tested in each of five (5) runs per concentration. Three reagent lots were tested for a total of 120 replicates per concentration. 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.

   The LoB was determined as 0.00 µmol/L, the LoD was determined as 0.004 µmol/L, and the LoQ was calculated to be 0.030 µmol/L.

7. Assay Cut-Off:
   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Method comparison studies were performed using a protocol based on CLSI EP09. Measurements of methotrexate in human serum (from patients treated with high-dose

methotrexate therapy) by the ARK Methotrexate II Assay on the Beckman Coulter AU680 were used to evaluate the agreement between the ARK Methotrexate II compared the predicate device ARK Methotrexate Assay, K111904 (immunoassay).

Methotrexate levels ranged from 0.054 to 1.168 µmol/L by the ARK methotrexate II Assay for one hundred twenty-three (123) specimens. Singlicate measurements of all specimens were used for method comparison.

Summary of the method comparison ARK Methotrexate II Assay vs ARK Methotrexate Assay data:

Parameter	**Range 0.054 to 1.168 µmol/L**
Number of Samples	123
Slope	0.98 (0.95 to 1.01)
y-intercept	-0.02 (-0.03 to -1.01)
Correlation Coefficient (r^2)	0.97 (0.96 to 0.98)

A method comparison was also performed against an LC-MS/MS comparator method. Methotrexate values ranged from 0.026 to 1.280. Statistics with confidence intervals from the Passing-Bablok regression are slope = 1.03 (1.00 to 1.06); y-intercept = 0.00 (-0.01 to 0.01); and correlation coefficient (r^2) = 0.98 (0.96 to 0.98).

2. Matrix Comparison:

Anticoagulated plasma and serum were evaluated to demonstrate equivalency of these matrices for measurement of methotrexate with the candidate device. Matched samples for serum and plasma from eight subjects were evaluated.

All the samples were tested on the Beckman Coulter AU680. Two calibrated runs were performed for samples from each subject. For each run, duplicate QCs and three (3) replicates of each sample matrix (Serum, Lithium Heparin, Sodium Heparin, Potassium EDTA) were performed. Every subject had a total of six (6) replicates for each sample matrix.

For the samples collected from the eight subjects, methotrexate was added to each matrix to give 1.00 µmol/L, and then serial dilutions were made with its own matrix/subject to give 0.50, 0.25, and 0.05 µmol/L respectively to span the measurement range. Serum matrix per subject served as the control.

The results of the analytical recovery of methotrexate in plasma ranged 94.0% to 102.1% of the recovery in serum.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable. Not typical for this type of assay.

2. Clinical Specificity:

   Not applicable. Not typical for this type of assay.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   The sponsor provided a discussion with balanced and representative literature discussing clinical use of methotrexate measurements.

D Clinical Cut-Off:

See expected values below

E 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. While the serum level may typically reach approximately 10 to 100 µmol/L in treatment of breast cancer (for example), concentrations may exceed 1000 µmol/L 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. For treatment of osteosarcoma, 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. Some centers follow ≤ 0.10 µmol/L.

From prescribing and other information: Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.

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 creatine	24 48 24

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs, for example vancomycin. 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.

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite, its molar ratio to methotrexate of up to approximately 100-fold, and relative insolubility versus the parent drug, possible nephrotoxicity due to precipitation of the metabolite in renal tubules 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. Elimination of DAMPA may take several days before it no longer interferes with the monitoring of methotrexate by immunoassay.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.