510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K233454

B Applicant

Roche Diagnostics

C Proprietary and Established Names

ONLINE TDM Methotrexate

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.

B Indication(s) 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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

All performance studies were conducted on the cobas c 503 Clinical Chemistry Analyzer.

IV Device/System Characteristics:

A Device Description:

The ONLINE TDM methotrexate assay consist of reagents: R1 (Anti-methotrexate antibody (rabbit monoclonal) and R3 (Methotrexate hapten conjugated to G6PDH).

B Principle of Operation:

The ONLINE TDM Methotrexate assay is a homogeneous enzyme-immunoassay. It 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.

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):	K233454	K111904
Device Trade Name	ONLINE TDM Methotrexate	ARK Methotrexate Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Methotrexate Assay is intended for the quantitative	Same

	determination of methotrexate. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.	
Sample Matrix	Human Serum or Plasma	Same
User Environment	Professional Clinical Laboratory: Prescription Use Only	Same
Measurement range	0.04 – 1.20 µmol/L	Same
General Device Characteristic Differences		
Reagent Components	Two (2) reagent system: Anti-Methotrexate Antibody/Substrate Reagent (R1) (contains rabbit monoclonal antibodies to Methotrexate) Enzyme Reagent (R3) (contains Methotrexate hapten conjugated to G6PDH)	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)

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*
- CLSI Guideline EP07- *Interference Testing in Clinical Chemistry – Third Edition*

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Performance was validated on the cobas c 503 instrument.

1. Precision/Reproducibility:

Precision was assessed based upon guidance in CLSI EP05-A3. Seven (7) human serum methotrexate samples and three (3) level of controls were used in the study. Each level of human serum samples and control was assayed in duplicate, twice a day over twenty days. Three reagent lots were assessed. A total of 84 determinations per sample per lot were obtained.

Mean methotrexate concentration, standard deviation (SD) and coefficients of variation (% CV) were calculated for within-run (repeatability) and intermediate precision (total).

Precision results for a representative reagent lot on a single instrument are summarized below:

Sample	N	Mean (µmol/L)	Repeatability		Mean (µmol/L)	Intermediate Precision	
			SD	%CV		SD	%CV
Controls							
Low	84	0.0863	0.00376	4.4	0.0737	0.00804	10.9
Mid	84	0.485	0.00421	0.9	0.487	0.00561	1.2
High	84	0.849	0.00567	0.7	0.841	0.00664	0.8
Patient Samples							
1	84	0.0872	0.00350	4.0	0.0752	0.00845	11.2
2	84	0.526	0.00427	0.8	0.526	0.00683	1.3
3	84	0.899	0.00648	0.7	0.889	0.00971	1.1
4	84	4.85	0.0491	1.0	4.91	0.0970	2.0
5	84	44.2	1.76	4.0	44.2	2.36	5.3
6	84	449	16.6	3.7	449	28.5	6.3
7	84	1334	41.8	3.1	1316	67.3	5.1

2. Linearity:

Linearity study was performed based upon guidance in CLSI EP06 2nd Edition.

A dilution series was prepared from human serum spiked with methotrexate and analyte-free human serum (sample Blank). Greater or equal to nine (9) dilutions were created by mixing the high human serum pool with the low human serum pool. Four (4) replicates were measured for each panel member using a single reagent lot on one cobas c 503 analyzers. The data was analyzed using a weight linear regression model.

The results support linearity across the claimed measuring interval of 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 measurement range of the candidate device. Samples were prepared by volumetric addition of methotrexate to human serum negative for methotrexate. Drug concentrations across the assay range were tested. Each samples was assayed in triplicate using three reagent lots for a total of nine 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	Recovery (%)
0.080	0.077	96.9
0.509	0.483	94.9
0.893	0.851	95.3

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 accuracy after dilution of samples was evaluated.

Evaluation of accuracy with elevated methotrexate levels:

Twelve (12) human serum samples were spiked with methotrexate and assayed in triplicate on cobas c 503 analyzer using a single reagent lot. The concentration of the samples ranged from 1.18 to 3167 µmol/L. The dilutions were created automatically using the instrument (automatic) or manually (manual) or both. Samples were diluted either 1:14 (automatic), 1:50 (automatic), 1:200 (manual), and 1:2800 (1:200 manual, 1:14 automatic). The recoveries were calculated of the mean from the assay versus the target value. The worst case of the lots showed results % deviation < 13%.

3. Analytical Specificity/Interference:

Potential interference substances were evaluated based upon guidance in CLSI EP07 3rd edition. Serum samples with low (~0.1 µmol/L) or high (~1.0 µmol/L) levels of methotrexate were spiked with endogenous or exogenous substances.

Five replicates of each sample and their respective serum controls were tested. The mean results of methotrexate were calculated and the percentage recoveries relative to the respective serum control mean results were determined.

Results that met the following criteria were determined to not interfere with the assay:

For samples ≤0.200 µmol/L, results within +/-0.0200 µmol/L of control mean results.

For samples >0.200 µmol/L, results within +/- 10.0% of control mean results.

The highest concentration tested that were determined to not interfere with the assay is described in the table below.

Exogenous Compound	Highest Concentration tested without significant interference
Acetaminophen	15.6 mg/dL
N-Acetylcysteine	15 mg/dL
Acetylsalicylic acid	3 mg/dL
Ampicillin-Na	7.5 mg/dL
Ascorbic acid	5.25 mg/dL
Cefoxitin	75 mg/dL
Cyclosporine	0.18 mg/dL
Doxycyclin	1.8 mg/dL
Heparin	3300 IU/L
Ibuprofen	21.9 mg/dL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Phenylbutazone	32.1 mg/dL
Rifampicin	4.8 mg/dL
Theophylline	6 mg/dL
5-Fluorouracil	9 mg/dL
5-Methyl-THF	45.9 mg/dL
6-Mercaptopurin	0.148 mg/dL
6-Methyl-5,6,7,8-tetrahydropterin-dihydrochlorid	25.4 mg/dL
7-Hydroxy methotrexate	9.35 mg/dL
Adriamycin	58 mg/dL
Carbamazepine	4.5 mg/dL
Chloramphenicol	7.8 mg/dL
Cisplatin	1.5 mg/dL
Cyclophosphamide	54.9 mg/dL
Cytosine	11.1 mg/dL
Digoxin	0.0039 mg/dL

Dihydrofolic acid	44.3 mg/dL
Disopyramide	1.68 mg/dL
Erythromycin	13.8 mg/dL
Folic acid	0.044 mg/dL
Furosemide	1.59 mg/dL
Gabapentin	2.67 mg/dL
Hydrochlorothiazide	0.113 mg/dL
Isoproterenol hydrochloride	0.00595 mg/dL
Leucovorin	142 mg/dL
Lidocaine	1.5 mg/dL
Naproxen	36 mg/dL
Phenobarbital	69 mg/dL
Phenytoin	6 mg/dL
Prednisolone	0.12 mg/dL
Prednisone	0.0099 mg/dL
Pyrimethamine	24.9 mg/dL
Sulfamethoxazole	40.5 mg/dL
Tetrahydrofolic acid	44.5 mg/dL
Triamterene	0.0585 mg/dL
Trimethoprim	4.2 mg/dL
Vancomycin	12 mg/dL
Vinblastine	81.1 mg/dL
Vincristine	82.5 mg/dL

Endogenous Compounds	**Highest Concentration tested without significant interference**
Albumin	7280 mg/dL
Conjugated Bilirubin	67 mg/dL
Unconjugated Bilirubin	75 mg/dL
Hemoglobin	1000 mg/dL
Immunoglobulin G	7530 mg/dL
Rheumatoid Factor	1134 IU/mL
Total Protein	12300 mg/dL
Cholesterol	441 mg/dL
Uric acid	31.3 mg/dL

The sponsor provided testing that supports the following claims in their labeling:

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).
Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).
Lipemia (Intralipid): No significant interference up to an L index of 1000.
There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1000 IU/mL.
Total protein: No significant interference from total protein in the concentration range of 2-12 g/dL.
As with any assay employing rabbit antibodies, the possibility exists for interference by human anti rabbit antibodies (HARA) in the sample, which could cause falsely lowered result.
In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

Cross reactivity to 7-Hydroxymethotrexate, the major metabolite
ONLINE TDM 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-N10-methylpteroic acid (DAMPA)

The sponsor claims that DAMPA can cross react with the ONLINE TMD Methotrexate Assay between 50-150%, and performed testing that was adequate to support this claim. The assay should not be used during possible compassionate therapy with glucarpidase (carboxypeptidase G2) that rapidly converts circulating methotrexate to DAMPA.

4. Assay Reportable Range:

   See linearity section (1 b.) above.

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

   ONLINE TDM Methotrexate is traceable to commercially available material.

6. Detection Limit:

   Limit of Detection (LoD) Limit of Quantitation (LoQ) studies were conducted based upon recommendations in CLSI Guideline EP 17-A2: *Protocols for Determination of Limits of Detection and Limits of Quantitation*. The study was performed on the cobas c 503 analyzer.

   For determination of LoB, one analyte-free serum 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. Data analysis is based on determination of the 95th percentile of the 60 measured values. The 95th percentile is the average of the 57th and 58th value.

For determination of LoD, five different human serum samples were spiked with different methotrexate concentrations to obtain samples within the range of LoB up to approximately 4x the specified LoB. The 5 samples were measured on three lots with 2-fold determination per run. Six runs distributed over 3 days on one instrument were performed. LoD was calculated according to EP17-A2.

Data analysis was based on determination of the 60 measured values of the five low analyte samples as follows:

LoD = LoB + 1.653 x SD total

For determination of LoQ, five different human serum samples were spiked with different methotrexate concentrations to obtain samples within the range of LoB up to approximately 2x the specified LoQ. The 5 samples were measured on three lots with 2-fold determination per run. Six runs distributed over 3 days on one instrument were performed. The LoQ was determined as the lowest concentration of analyte which can be quantified with a total error of no more than 30%.

The studies support the following claimed LoB, LoD, and LoQ:
The LoB was claimed to be 0.0250 µmol/L.
The LoD was claimed to be 0.0350 µmol/L.
The LoQ was claimed to be 0.0400 µmol/L.

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

The sponsor conducted a method comparison study of the candidate device against a validated comparator. A total of 105 samples were analyzed including 38 serum samples and 67 plasma samples from methotrexate patients. The concentration range of the samples was 0.043 to 1.190 µmol/L. The performance summarized in the table below describes both serum and plasma.

Number of Samples	105
Slope	1.03 (1.014 to 1.049)
y-intercept	-0.00 (-0.002 to 0.001)
Correlation Coefficient (r)	0.99

2. Matrix Comparison:

Anticoagulated plasma and serum were evaluated to demonstrate equivalency of these matrices for measurement of methotrexate with the ONLINE TDM Methotrexate. Matched samples for serum and plasma from at least 50 subjects were evaluated.

All the samples were tested using 1 reagent lot on the cobas c 503 analyzer. A method comparison was performed by taking the serum matrix per subject as the reference (control).

The results of the linear regression are shown below:

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

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

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

   Not applicable.

D Clinical Cut-Off:

See expected values below.

E Expected Values/Reference Range:

The following is included in the package insert:

Methotrexate serum concentrations are highly variable depending on dosage and route of administration in different indications, pharmacokinetics such as hepatic and renal function, coadministration of other drugs and other clinical factors. To decrease the risk of severe methotrexate toxicity, the rescue agent leucovorin is administered in intermediate- and high-dose methotrexate therapy. Administration is guided by methotrexate concentrations and the leucovorin dose must be increased with increased MTX concentrations. According to the cited prescribing information of Leucovorin calcium injection, the following MTX thresholds are the basis for leucovorin dosing:

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

In high dose MTX therapy, plasma concentrations above 1000 µmol/L can be reached, for instance in osteosarcoma patients.1 Methotrexate monitoring and leucovorin administration should be continued until methotrexate plasma target concentrations are reached (typically, below 0.1 or 0.2 µmol/L).

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and / or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.

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.