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Introduction

ABSTRACT

Background

Monitoring methotrexate (MTX) levels is essential during high-dose methotrexate therapy. Acute lymphoblastic leukemia, lymphoma, osteosarcoma, breast cancer, and head and neck cancer are the prominent indications. Serum levels may reach 1000 µmol/L or more, and renal toxicity is a risk. Leucovorin supports folate metabolism while MTX levels decline to safe concentrations. Ability to measure MTX accurately at 0.05 µmol/L enables clinical determination of non-toxic status.

Methods

The analytical performance of a new ARK™ Methotrexate Assay, a homogenous enzyme immunoassay for quantifying MTX in human serum or plasma, was evaluated on the Roche/Hitachi 917 system with a six point calibration curve (0.00 to 1.20 µmol/L) and six-level (0.07, 0.40, 0.80, 5, 50, and 500 µmol/L) quality controls. Performance testing included precision, limit of quantitation, linearity, endogenous interference, specificity, proficiency samples, and method comparison to Abbott TDx® MTX II Assay.

Results presented in this poster update design verification studies. ARK Diagnostics gratefully acknowledges the assistance of Johns Hopkins Medical Institution Laboratory (Baltimore, MD), MedTox Laboratories (St Paul, MN), Stanford University Medical Center (Stanford, CA) and William Beaumont Hospital (Royal Oak, MI).

Precision

Precision was determined as described in CLSI/NCCLS Protocol EP5-A2. Each level of the ARK Methotrexate Control 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.

Sample	N	Mean (µmol/L)	Within Run		Between Day		Total	
			SD	CV(%)	SD	CV (%)	SD	CV (%)
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
5*	160	4.8	0.15	3.1	0.13	2.8	0.20	4.2
50*	160	49	1.36	2.8	2.32	4.8	2.72	5.6
500*	160	476	15.17	3.2	30.75	6.5	34.66	7.3

*Samples were diluted in ARK Methotrexate Dilution Buffer. Mean result and SD were multiplied by the dilution factor.

Limit of Quantitation and Recovery

Limit of quantitation was evaluated according to CLSI/NCCLS EP17-A. Pooled human serum was supplemented with methotrexate to give concentrations of 0.02, 0.03, 0.04, and 0.05 µmol/L. Drug concentrations across the measurement range (0.06, 0.10, 0.30, 0.60, and 1.00 µmol/L) were tested, six replicates. An overall mean percentage recovery was 102.1%.

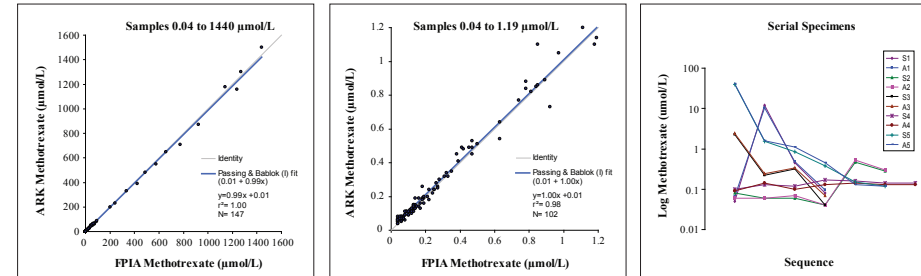
Criterion	MTX (µmol/L)	N
Limit of Blank (LoB); $\mu B + 1.645 SD$, where $SD = 0.005$	0.01	60
Limit of Detection (LoD); $LoB + 1.652 SD$, where $SD = 0.005$	0.02	60
Limit of Quantitation (LoQ); $LoQ - 2 SD > LoD$	0.04	40

Target (µg/mL)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
0.06	0.067	0.008	12.2	111.1
0.10	0.100	0.006	6.3	100.0
0.30	0.295	0.010	3.6	98.3
0.60	0.613	0.029	4.7	102.2
1.00	0.988	0.090	9.1	98.8

Mean percent recovery: 102.1

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. Plasma specimens were collected serially from five patients treated with methotrexate. The concentration of methotrexate was determined by FPIA (S) and the ARK Methotrexate Assay (A). The serial pattern in methotrexate followed clinically was the same for both methods.



Specificity

Crossreactivity to 7-Hydroxymethotrexate, the major metabolite

The ARK Methotrexate Assay did not crossreact with the major metabolite 7-OH-MTX in the presence of methotrexate at either 0.05 or 0.50 µmol/L in serum.

7-OH methotrexate (µmol/L)	Serum Control MTX (µmol/L)	Test MTX (µmol/L)	Cross Reactivity (%)
5.0	0.04	0.05	0.02
50.0	0.46	0.48	0.05

The ARK Methotrexate Assay crossreacts substantially with the minor metabolite DAMPA (2,4-diamino-N¹⁰-methylptericoic acid), 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. The ARK Methotrexate Assay crossreacts slightly with triamterene (2.3%) and trimethoprim (0.5%), 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.

Crossreactivity to folate analogs, other compounds and endogenous interference

The ARK Methotrexate Assay did not crossreact ($\leq 0.01\%$) with folate analogs or other compounds at ≥ 1000 µmol/L as tested. 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. Measurement of methotrexate by the ARK Methotrexate Assay was not affected by the presence of interfering substances at the levels tested.

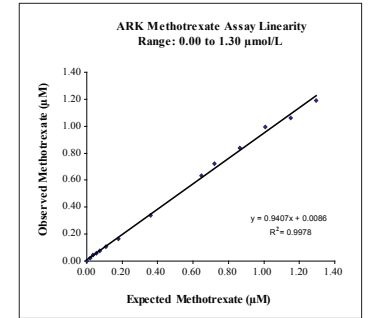
Compound	Tested (µmol/L)
Adriamycin	1000
Cyclophosphamide	1500
Cytosine	1000
Dihydrofolic Acid	1000
DL-6-Methyl-5,6,7,8-Tetrahydropterine	1000
Folic Acid	1000
Folinic Acid (leucovorin)	1000
5-Fluorouracil	3000
6-Mercaptopurine	1000
5-Methyltetrahydrofolic acid	1000
Prednisolone	1000
Pyrimethamine	1000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

Compound	Interferent Concentration	Methotrexate (~ 0.05 µmol/L)		Methotrexate (~ 0.50 µmol/L)
		Serum Control	Test	Control (%)
Albumin	12 g/dL	0.05	0.06	92.8
Bilirubin - conjugated	70 mg/dL	0.05	0.06	105.5
Bilirubin - unconjugated	70 mg/dL	0.05	0.06	106.9
Cholesterol	400 mg/dL	0.05	0.06	105.4
Gamma-Globulin	12 g/dL	0.05	0.06	105.5
Hemoglobin	1000 mg/dL	0.04	0.05	93.2
Intralipid®	500 mg/dL	0.05	0.05	105.1
Rheumatoid Factor	1100 IU/mL	0.05	0.06	96.1
Triglycerides	749 mg/dL	0.04	0.04	91.4
Uric Acid	30 mg/dL	0.05	0.04	102.8

Linearity and Proficiency

Linearity studies were performed as suggested in CLSI/NCCLS Protocol EP6-A. Samples containing methotrexate were prepared proportionally in pooled human serum. Regression of assayed methotrexate concentrations was linear throughout the range. TDM Survey Samples from Heath Controls (UK NEQAS: United Kingdom National External Quality Assessment Scheme, LGC Standards, Middlesex, U.K.) and College of American Pathologists (CAP ; Northfield, IL) were evaluated. Tests by the ARK assay were considered within the consensus range for the predicate FPIA device.

MTX Sample ID	FPIA Consensus		ARK Methotrexate Assay	
	Mean (µmol/L)	SD	Mean n=6 (µmol/L)	SD
Heath Control (UK NEQAS)				
0610	10.79	1.39	11.40	1.08
0710	0.27	0.02	0.26	0.004
0810	0.41	0.03	0.46	0.010
1010	0.03	0.02	0.02	0.000
1110	3.75	0.30	3.50	0.09
1210	0.10	0.02	0.09	0.005
College of American Pathologists (CAP) Survey				
CHM01	35.17	2.56	32.17	0.98
CHM02	8.12	0.46	7.67	0.43



Conclusions

The ARK Methotrexate Assay provided quantitative measurement MTX in serum and plasma on the Roche/Hitachi 917 and correlated with TDx Methotrexate II Assay (FPIA). Its homogeneous enzyme immunoassay technology is well-suited for routine TDM of MTX on automated clinical laboratory systems.

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.

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.

Regulatory Status

CE Marked - Europe
Licensed in Canada
Pending FDA 510(k) clearance - USA