Performance of the ARK Diagnostics, Inc., ARK™ Methotrexate Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System

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Introduction

- The ARK Methotrexate Assay quantitatively determines the concentration of methotrexate in human serum or plasma on automated clinical chemistry analyzers.
- Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis.[1]
- HDMTX or high-dose methotrexate therapy (defined as greater than 500 mg/m^2), is used for adult and childhood cancers. Due to its toxicity, especially on kidneys, HDMTX can have significant morbidity and mortality effects and lead to acute kidney injury (AKI) in 2-12% of patients.[2]
- Methotrexate monitoring is part of the pharmacokinetically guided treatment protocols to ensure appropriate therapeutic levels of less than 0.05 – 0.1 µmol/L, and avoid possible toxic effects of the treatment.[2]

Assay Method Overview

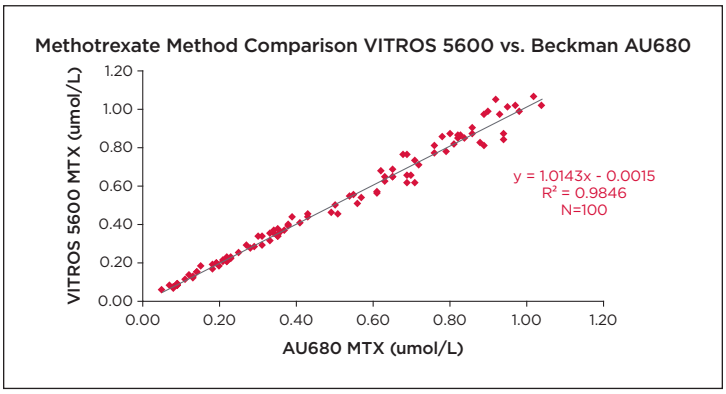
- The performance of the ARK Methotrexate Assay was assessed on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System.
- The assay was conducted using 8.5 µL of patient sample and the two ARK Methotrexate Assay reagents.
- Two-point rate measured at 340nm is converted to concentration using a Logit/Log 4 calibration model. Enzyme activity (rate) is directly related to the concentration of Methotrexate in the patient specimen.

Assay Enzymatic Reaction Scheme

- The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between methotrexate present in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent.
- Antibody binding to G6PDH decreases enzyme activity, while binding of methotrexate from the specimen to the antibody reduces antibody bound to G6PDH, thereby increasing enzyme activity. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH that is measured spectrophotometrically as a rate of change in absorbance.

Method Comparison

- A split sample comparison was conducted with 100 serum and samples (0.05 – 1.04 µmol/L Methotrexate) using the VITROS 4600/5600 Systems and the Beckman Coulter AU680 Clinical Chemistry Analyzer.
- The VITROS 4600 and VITROS 5600 Systems showed excellent correlation with the AU680 Analyzer.



Data shown from VITROS 5600 Integrated System

Precision

- Total within-laboratory precision was evaluated in accordance with CLSI EP15-A3 by evaluating Methotrexate controls at 0.07 µmol/L, 0.40 µmol/L and 0.80 µmol/L, 4 replicates twice per day for 5 days, for a total of 40 replicates.
- Precision components generated using Analyze-it® software are shown for the VITROS 4600 and VITROS 5600 Systems.

ANOVA Components	VITROS 4600			VITROS 5600		
	QC Low	QC Mid	QC High	QC Low	QC Mid	QC High
Mean	0.076	0.417	0.847	0.075	0.418	0.857
Between-Day SD	0.001	0.007	0.016	0.005	0.002	0.011
Between-Run SD	0.006	0.006	0.005	0.003	0.000	0.000
Within-Run SD (Repeatability)	0.007	0.010	0.032	0.004	0.012	0.038
Total SD (Within-Lab)	0.009	0.014	0.036	0.007	0.013	0.039
Total % CV	11.8%	3.4%	4.3%	9.3%	3.1%	4.6%
Within Run %CV	9.2%	2.4%	3.8%	5.3%	2.9%	4.4%

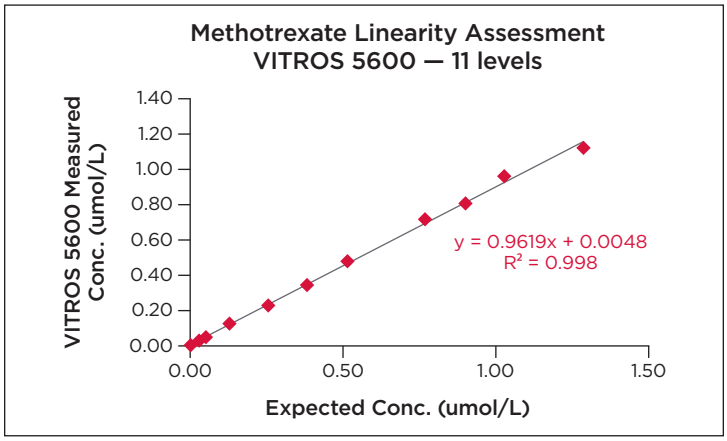
Limit of Quantitation

- The Limits of Blank (LoB), Detection (LoD) and Quantitation (LoQ) were determined in accordance with CLSI EP17-A2.
- LoB and LoD were determined by the non-parametric and parametric methods, respectively as described in the CLSI guideline.
- LoQ was determined by the precision profile approach as the predicted concentration at which the precision estimate was ≤ 20% CV.
- The values reported are the most conservative across the VITROS 4600 and VITROS 5600 Systems.

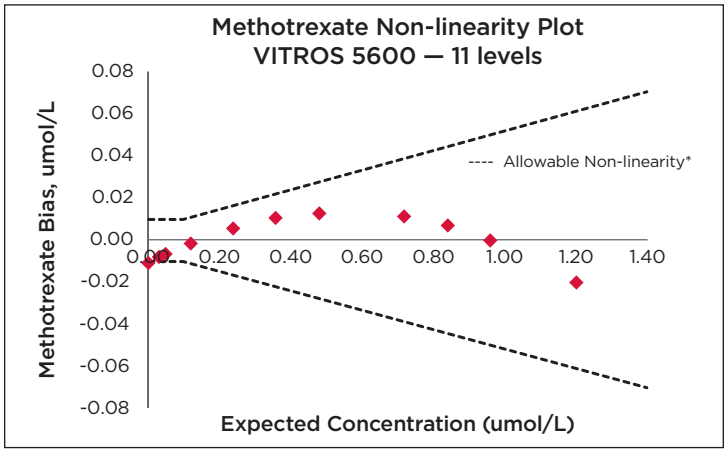
VITROS System	LoB (umol/L)	LoD (umol/L)	LoQ (umol/L)
4600 /5600	0.005	0.023	0.04

Linearity

- The linearity of the ARK Methotrexate assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated in accordance with CLSI EP06-A.
- The assessment was conducted using an 11 level analyte supplemented pooled serum admixture series. The observed linear range was 0.029 – 1.122 µmol/L.



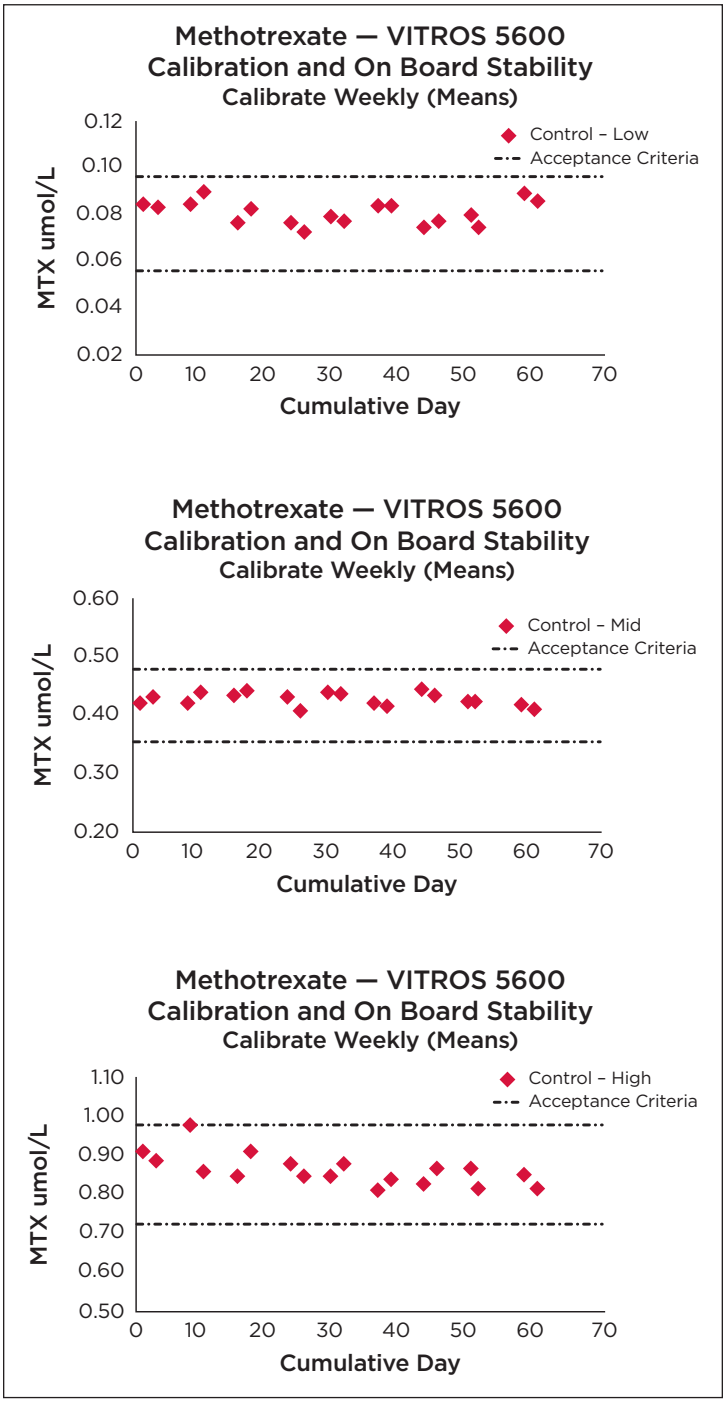
Data shown from VITROS 5600 Integrated System



*Allowable bias = 0.01 umol/L at [MTX] ≤ 0.10umol/L, <5% at [MTX] > 0.10umol/L

Onboard Reagent Stability

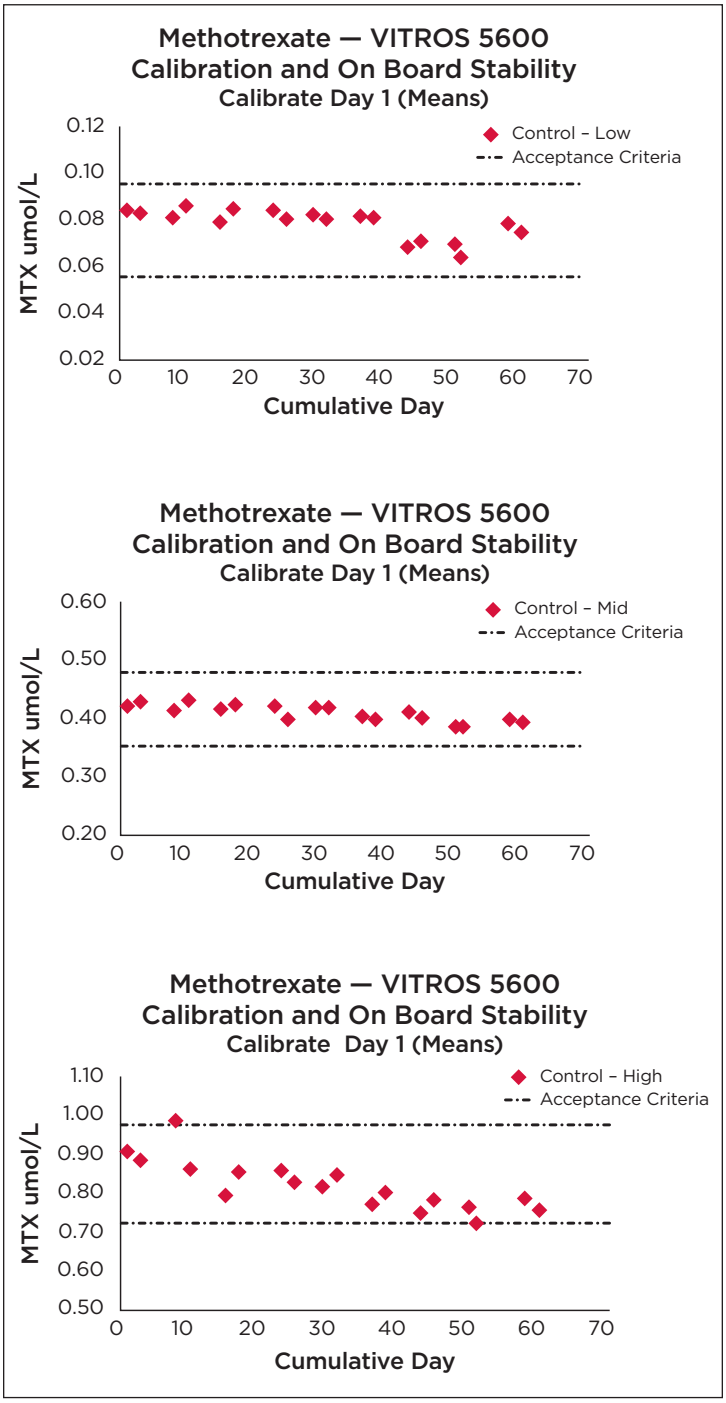
- The stability of the ARK Methotrexate reagents while stored onboard the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated.
- ARK MTX Controls were used as the test fluids with 2 replicates per timepoint. Mean values at each timepoint were plotted (18 timepoints, 36 total replicates).
- Calibration was done at 7 day (weekly) intervals.
- Results indicate that the MTX reagent onboard stability is at least 60 days.



Data shown from VITROS 5600 Integrated System

Calibration Stability

- The calibration stability of the ARK Methotrexate assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated.
- A single calibration was done on day 1 of testing.
- ARK MTX Controls were used as the test fluids over 18 test days (60 cumulative days). Mean values at each timepoint were plotted (18 timepoints, 36 total replicates).
- Results indicate that the MTX assay calibration stability is at least 25 days based on the most conservative result.



Data shown from VITROS 5600 Integrated System

High Sample Dilution

- High Sample Dilution was evaluated manually and using auto dilution for the ARK Methotrexate assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System.
- Methotrexate prediluted simulated serum samples were tested to evaluate manual dilution, and the same samples were prediluted to an appropriate concentration to allow the analyzer to perform the last 1:10 dilution.
- Results indicate less than a 10% bias between manual and auto dilution.

Manual Dilution VITROS 5600						
µmol/L	5.00	50.00	500.00	2.00	20.00	200.00
Mean (µM)	0.52	0.54	0.55	0.21	0.20	0.20
x dilution factor	5.17	54.33	548.89	2.08	19.59	197.08
SD	0.004	0.013	0.009	0.011	0.004	0.006
CV %	0.8	2.5	1.6	5.4	2.2	3.0
N	6	6	6	6	6	6
% Recovery	103.3	108.7	109.8	103.8	98.0	98.5

Auto Dilution VITROS 5600						
µmol/L	5.00	50.00	500.00	2.00	20.00	200.00
Mean (µM)	5.13	5.01	5.16	2.06	1.91	2.04
x dilution factor	5.13	50.14	515.99	2.06	19.08	203.50
SD	0.100	0.161	0.089	0.049	0.077	0.056
CV %	1.9	3.2	1.7	2.4	4.0	2.8
N	6	6	6	6	6	6
% Recovery	102.5	100.3	103.2	103.1	95.4	101.8
% Diff. from Manual	-0.79	-7.71	-5.99	-0.74	-2.61	3.26

Data shown from VITROS 5600 Integrated System

Conclusions

The performance of the ARK Diagnostics, Inc., ARK Methotrexate Assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated. The assay exhibited the following:

- ✓ Excellent correlation with the Beckman AU680 Clinical Chemistry Analyzer
- ✓ Excellent Within-Lab precision and low end sensitivity
- ✓ Onboard reagent stability to at least 60 days
- ✓ Calibration stability to 23 days
- ✓ Comparable results when using either the Auto Dilution function or manual dilution on VITROS systems up to a 1:10 dilution

Sources:

1. https://www.rxlist.com/trexall-drug.htm#description, Accessed June 4, 2018
2. Howard, Scott C., et al. *Preventing and managing Toxicities of High-Dose Methotrexate* The Oncologist, 2016; 21:1-12.