Performance Characteristics of Three Assays for the Therapeutic Drug Monitoring of Methotrexate

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Abstract

Background: Methotrexate (MTX) at high-dosage is used in the treatment of malignancies, while low-dose therapy is commonly used for nonmalignant diseases such as psoriasis. MTX toxicity can be life-threatening occurring at both high- and low-dose therapies. Management of MTX toxicity by leucovorin rescue includes monitoring plasma MTX concentrations to very low (<0.05 μmol/L) or undetectable levels. Due to growing concerns for the future unavailability of the TDx/FLx analyzer (Abbott Diagnostics), we evaluated MTX reagents from ARK diagnostics on the Beckman AU400 analyzer and performed a comparison on the Vitros 5.1 Fusion (Ortho Clinical Diagnostics), using the SIEMENS Syva® Emit® MTX assay against the TDx/FLx analyzer for comparable performance.

Objective: Evaluate the performance characteristics of three immunoassays on three separate analyzers for the quantitative measurement of plasma methotrexate.

Methods: Imprecision studies were performed by analyzing the different levels of quality control materials over a three day period. Similarly, sensitivity and linearity were evaluated in duplicate for each assay. For method comparison, 58 plasma samples from patients undergoing therapeutic drug monitoring (TDM) were tested by all methods with the TDx/FLx as the reference method.

Results: Within- and between-run imprecision were acceptable, with total CV’s ranging from 5.3% - 10.2%. Linearity and limit of quantitation were concordant with the manufacturers’ determinations. Based on these results, all 3 assays can be used to analyze patient samples with methotrexate concentrations ranging from 0.02-1000 μmol/L (Abbott), 0.3-2600 μmol/L (Ortho) and 0.05-1200 μmol/L (ARK Diagnostics). Method comparison results by linear regression had slopes of 1.03 (TDx/FLx vs. Fusion) and 1.147 (TDx/FLx vs. AU400), demonstrating a slight positive bias. Nevertheless, both methods correlated well to the TDx/FLx (correlation coefficient = 0.95).

Conclusion: The Fusion and AU400 assays have inherent limitations at the lower range with the Syva® Emit® and ARK MTX reagents respectively, giving higher results compared to the TDx/FLx. The ARK MTX reagent was most comparable to the TDx/FLx at the lower range demonstrating a sensitivity of 0.05 μmol/L compared to 0.02 μmol/L for the TDx/FLx and is an acceptable alternative to the Abbott TDx/FLx for MTX TDM.

Introduction

MTX is an antifolate drug commonly used as an antineoplastic agent. High-dose MTX is used in the treatment of certain malignancies including leptomeningeal metastases, systemic non-Hodgkin lymphoma, acute lymphoblastic leukemia and osteosarcoma. Low-dose MTX is commonly used in the treatment of various nonmalignant disorders including rheumatoid arthritis (RA), psoriasis and ectopic pregnancies. As an antifolate drug, MTX acts by interfering with folate metabolism through inhibition of key enzymes in this process such as dihydrofolate reductase. This results in the depletion of intracellular pools of reduced folates required for DNA synthesis. Rapidly dividing malignant cells which require greater amounts of reduced folates are preferentially affected by MTX, resulting in the cessation of DNA synthesis and eventually cell death.

High-dose intravenous infusions of MTX are usually followed by leucovorin (a formulation of reduced folate) rescue to neutralize the toxic effects of MTX on normal cells. Management of MTX toxicity by leucovorin rescue includes monitoring plasma MTX concentrations to very low (<0.05 μmol/L) or undetectable levels. At ARUP Laboratories, we currently use the Abbott TDx/FLx Methotrexate II assay on the TDx/FLx analyzer. However, there are growing concerns for the future unavailability of the TDx/FLx analyzer (Abbott Diagnostics). We therefore evaluated MTX reagents from ARK diagnostics on the Beckman AU400 analyzer and performed a comparison on the Vitros 5.1 Fusion (Ortho Clinical Diagnostics), using the SIEMENS Syva® Emit® MTX assay against the TDx/FLx analyzer for comparable performance.

Materials and Methods

Specimen Selection: Plasma samples sent to ARUP Laboratories for routine therapeutic drug monitoring of methotrexate therapy were analyzed.

Validation of Two Quantitative Methotrexate Assays: Linearity and sensitivity (limit of detection) was determined for each assay, with samples analyzed in duplicates. The reportable range of each assay was verified by calibrator recovery spiking studies and testing of samples with methotrexate results spanning the respective reportable ranges. Imprecision studies were performed by assaying two to three levels of quality control materials, five times daily, over a three day period. For method comparison, MTX concentrations were quantified using the following assays and analyzers: (i) The Abbott TDx/FLx Methotrexate II assay on the TDx/FLx analyzer as the reference method. This assay utilizes a fluorescence polarization immunoassay technique; (ii) Siemens Syva® Emit® Methotrexate assay on the Vitros 5.1 Fusion from Ortho Clinical Diagnostics. This assay utilizes a homogeneous enzyme immunoassay technique. Data analysis was computed by GraphPad Prism.

Results

Table 1: Result summary of validation studies for two different methotrexate assays performed on the indicated automated analyzers and compared to the TDx/FLx methotrexate assay as the reference method. ASR - Analytical measurement range; CRR - Clinical reportable range; CV - Coefficient of variation; LOD - Limit of detection.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>TDx/FLx Methotrexate</th>
<th>Syva® Emit® Methotrexate</th>
<th>ARK Methotrexate</th>
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<tr>
<td>Linearity</td>
<td></td>
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<tr>
<td>ASR</td>
<td>0.02 – 10 μmol/L</td>
<td>0.02 – 10 μmol/L</td>
<td>0.05 – 10 μmol/L</td>
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<tr>
<td>CRR</td>
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<td>0.3 – 2600 μmol/L</td>
<td>0.05 – 1200 μmol/L</td>
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<tr>
<td>Line Equation</td>
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<td>y = 0.96x + 0.001, R ² = 0.998</td>
<td>y = 0.94x + 0.009, R² = 0.998</td>
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<tr>
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<tr>
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<tr>
<td>Mean Percent Recovery (%)</td>
<td>102.4%</td>
<td>102.1%</td>
<td>102.7%</td>
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</table>

Figure 1: Comparison of the Syva® Emit® MTX with the TDx/FLx MTX assay using Windows 10 (solid line), the dashed line represents the ideal situation (y=x). (A) Regression analysis (solid line), the dashed line represents the ideal situation (y=x). (B) Parameters obtained from D’Agostino regression analysis in (A). N, the standard error of the estimate; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to y=x.

Figure 2: Comparison of the ARK Methotrexate with the TDx/FLx MTX assay using (A) D’Agostino regression analysis (solid line), the dashed line represents the ideal situation (y=x). (B) Parameters obtained from D’Agostino regression analysis in (A). N, the standard error of the estimate; * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 compared to y=x.

Conclusions

- The Syva® Emit® and ARK methotrexate assays have inherent limitations at the lower range, giving higher results compared to the TDx/FLx.
- The Syva® Emit® assay had less bias when compared to the TDx/FLx with a slope of 1.03, versus 1.147 when the ARK methotrexate assay is compared to the TDx/FLx.
- However, the ARK methotrexate assay was most comparable to the TDx/FLx at the lower range demonstrating a sensitivity of 0.05 μmol/L compared to 0.02 μmol/L for the TDx/FLx.
- The ARK methotrexate assay is therefore an acceptable alternative to the Abbott TDx/FLx for methotrexate therapeutic drug monitoring.

References


Acknowledgments

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