MONITORING METHOTREXATE BY ARK™ IMMUNOASSAY

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Introduction

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 control. Performance testing included precision, limit of quantitation, linearity, endogenous interference, specificity, proficiency samples, and method combination to Abbott TDx™ MTX Assay.

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

Method Comparison
Correlation studies were performed using CLSI/NCCCLS Protocol EP9-A2. Results from the ARK Methotrexate Assay were compared with results from Fluorescence Polarization Immunoassay method (Immunoplate FPIA). Methotrexate concentrations by FPIA ranged 0.04 to 1440 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.

Limit of Quantitation and Recovery

Limit of quantitation was evaluated according to CLSI/NCCCLS EP17-A. Pooled human serum was supplemented with methotrexate to give concentrations of 0.03, 0.04 and 0.05 μmol/L. Drug concentrations across the measurement range (0.06, 0.10, 0.35, 0.60, and 1.00 μmol/L) were tested, six replicates. An overall mean percentage recovery was 102.1%.

Precision
Precision was determined as described in CLSI/NCCCLS 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 an hour. The within run, between run, total CV, and percent CVs were calculated.

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. The ARK Methotrexate Assay did not crossreact with the major metabolite 7-OH-MTX in the presence of methotrexate.

Crossreactivity to folate analogs, other compounds and endogenous interference
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.

Linearity

Linearity studies were performed as suggested in CLSI/NCCCLS Protocol EP6-A. Samples containing methotrexate were prepared proportionally to pooled human serum. Regression of assayed methotrexate concentrations was linear throughout the range. TDM Survey Samples from Health 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.

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 homogenous enzyme immunoassay technology is well suited for routine TDM of MTX on automated clinical laboratory systems.

Intended Use

The ARK Methotrexate Assay is a homogenous 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

Table: ARK Methotrexate Assay Linearity

<table>
<thead>
<tr>
<th>Composed</th>
<th>Calibration Concentration (μmol/L)</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>0.00</td>
<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1.00</td>
<td>100.0</td>
<td>95.6</td>
</tr>
<tr>
<td>Crossreactivity</td>
<td>7-OH-MTX</td>
<td>0.0002</td>
<td>0.100</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>1.00</td>
<td>100.0</td>
<td>95.6</td>
</tr>
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</table>

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