Overview

Useful For
Determining whether methotrexate is being cleared appropriately and verifying that a nontoxic concentration has been attained following therapy

Method Name
Immunoassay

NY State Available
Yes

Specimen

Specimen Type
Serum

Advisory Information
For patients that have received glucarpidase (carboxypeptidase G2) as a high-dose methotrexate rescue therapy, order MTXSG / Methotrexate Post Glucarpidase, Serum.

Shipping Instructions
Ship specimen in amber vial to protect from light.

Specimen Required

Supplies: Amber Frosted Tube, 5 mL (T192)

Collection Container/Tube:

Preferred: Serum gel

Acceptable: Red top

Submission Container/Tube: Amber vial

Specimen Volume: 0.5 mL

Collection Instructions:

1. Methotrexate is sensitive to fluorescent light; avoid prolonged exposure of specimen to direct light.

2. Serum gel tubes should be centrifuged within 2 hours of collection. Protect from light.

3. Red-top tubes should be centrifuged and serum aliquoted into amber vial within 2 hours of collection.

Forms
If not ordering electronically, complete, print, and send a Therapeutics Test Request (T831) with the specimen.

Specimen Minimum Volume
Test Definition: MTHX
Methotrexate, S

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0.25 mL

**Reject Due To**

<table>
<thead>
<tr>
<th>Gross hemolysis</th>
<th>Reject</th>
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**Specimen Stability Information**

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<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
<th>Special Container</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Refrigerated (preferred)</td>
<td>14 days</td>
<td>LIGHT PROTECTED</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>28 days</td>
<td>LIGHT PROTECTED</td>
</tr>
<tr>
<td></td>
<td>Ambient</td>
<td>72 hours</td>
<td>LIGHT PROTECTED</td>
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**Clinical and Interpretive**

**Clinical Information**

Methotrexate is an antineoplastic agent that inhibits DNA synthesis. The medication exerts its effects through competitive inhibition of the enzyme dihydrofolate reductase thus decreasing the concentrations of tetrahydrofolate essential to the methylation of pyrimidine nucleotides and consequently the rate of pyrimidine nucleotide and ultimately DNA synthesis.

Methotrexate is used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. Methotrexate is effective against malignancies characterized by rapid cell proliferation. Intermediate to high doses of methotrexate with leucovorin (citrovorum-factor or folinic acid) rescue to salvage nontumor cells have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin lymphoma, lung, and breast cancer.

Methotrexate has the potential for serious toxicity. Patients undergoing methotrexate therapy are closely monitored so that toxic effects are detected promptly.

**Reference Values**

Nontoxic drug concentration after 72 hours: <0.1 mcmol/L

**Interpretation**

Serum concentrations of methotrexate are commonly monitored during high-dose therapy (>50 mg/m[2]) to identify the time at which active intervention by leucovorin rescue should be initiated. Criteria for serum concentrations indicative of a potential for toxicity after single-bolus, high-dose therapy are as follows:

- Methotrexate >10 mcmol/L 24 hours after dose
- Methotrexate >1 mcmol/L 48 hours after dose
- Methotrexate >0.1 mcmol/L 72 hours after dose

**Cautions**

**Important:** Specimens from patients who have received glucarpidase (carboxypeptidase G2) as a high-dose methotrexate rescue therapy should not be tested with this immunoassay. Methotrexate concentrations should be performed using the liquid chromatography-tandem mass spectrometry method to avoid the reporting of falsely
Elevated methotrexate values due to an interference that could confuse the efforts of the glucarpidase therapy. After glucarpidase therapy, it can take at least 5 to 7 days before accurate measurements of serum methotrexate can be obtained using an immunoassay.

**Clinical Reference**


**Performance**

**Method Description**

The ARK Methotrexate Assay is a homogeneous immunoassay based on competition between drug in the specimen and methotrexate labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding to the antibody reagent. As the latter binds antibody, enzyme activity decreases. In the presence of drug from the specimen, enzyme activity increases and is directly proportional to the drug concentration. Active enzyme converts the coenzyme nicotinamide adenine dinucleotide (NAD) to NADH (the reduced form of NAD) that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenzyme NAD functions only with the bacterial enzyme used in the assay. (Package insert: ARK Methotrexate reagent, ARK Diagnostics, Inc., Fremont, CA)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Sunday; Continuously

**Analytic Time**

Same day/1 day

**Maximum Laboratory Time**

1 day

**Specimen Retention Time**

1 week

**Performing Laboratory Location**

Rochester

**Fees and Codes**

**Fees**

- Authorized users can sign in to [Test Prices](#) for detailed fee information.
- Clients without access to Test Prices can contact [Customer Service](#) 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact [Customer Service](#).
Test Definition: MTHX
Methotrexate, S

Test Classification
This test has been cleared, approved or is exempt by the U.S. Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information
80204

LOINC® Information

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<th>Test ID</th>
<th>Test Order Name</th>
<th>Order LOINC Value</th>
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<td>Methotrexate, S</td>
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