Test Definition: MTHX

Methotrexate, Serum

Overview

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

Method Name
Enzyme Multiplied Immunoassay (EMIA)

NY State Available
Yes

Specimen

Specimen Type
Serum

Ordering Guidance
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 (T915)
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. Within 2 hours of collection, centrifuge the specimen.
3. For serum gel tubes, aliquot serum into a plastic vial within 2 hours of collection. Keep protected from light.
4. For red-top tubes, aliquot serum into a plastic vial immediately. Keep protected from light.

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

Specimen Minimum Volume
0.25 mL

Reject Due To

Gross hemolysis	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Serum	Refrigerated (preferred)	14 days	LIGHT PROTECTED
	Ambient	72 hours	LIGHT PROTECTED
	Frozen	28 days	LIGHT PROTECTED

Clinical & 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

1. Snozek CLH, McMillin GA, Moyer TP. Therapeutic drugs and their management. In: Burtis CA, Ashwood ER, Bruns D. Tietz Textbook of Clinical Chemistry. 5th ed. WB Saunders Company; 2012:1057-1108
2. Cadman EC, Durivage HJ: Cancer chemotherapy: alkylating agents. In: Wilson JD, Braunwald E, Isselbacher KJ, et al. Harrison's Principles of Internal Medicine. 12th ed. McGraw-Hill Book Company; 1991:1592-1594
3. LaCasce AS. Therapeutic use and toxicity of high-dose methotrexate. UpToDate; Updated August 1, 2024. Accessed February 18, 2025. Available at www.uptodate.com/contents/therapeutic-use-and-toxicity-of-high-dose-methotrexate

Performance

Method Description

The ONLINE TDM MTX assay is a homogeneous enzyme multiplied immunoassay (EMIA). It is a two-reagent system used for the detection of methotrexate in serum and plasma. In this technology, drug hapten attached to the glucose-6-phosphate dehydrogenase (G6PD) enzyme serves as the binding partner to anti-methotrexate antibody. A competitive reaction to a limited amount of specific anti-methotrexate antibody takes place between the enzyme bound hapten and free methotrexate in the sample. Enzyme activity is reduced with bound antibody. Only active enzymes reduce nicotinamide adenine dinucleotide (NAD[+]) to NADH. The rate of NADH formation during the reaction correlates to the methotrexate concentration and is measured photometrically.(Package Insert: ONLINE TDM Methotrexate; Roche Diagnostics; 03/2025)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

Same day/1 day

Specimen Retention Time

1 week

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. For assistance, contact Customer Service.

Test Classification

This test has been cleared, approved, or is exempt by the US 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

Test ID	Test Order Name	Order LOINC® Value
MTHX	Methotrexate, S	14836-1

Result ID	Test Result Name	Result LOINC® Value
MTHX	Methotrexate, S	14836-1