# Methotrexate

## Purpose

This procedure provides instructions for performing Methotrexate on plasma or serum in Children's Minnesota Laboratory- Minneapolis on Abbott Alinity c systems.

## Policy Statements

This procedure applies to all chemistry staff responsible for analyzing and reporting Methotrexate in serum or plasma on the Abbott Alinity c systems.

## Principle

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 that is measured spectrophotometrically as a rate of change in absorbance. Endogenous serum G6PDH does not interfere with the results because the coenyzme NAD functions only with the bacterial enzyme used in the assay.

## Clinical Significance

Methotrexate is an anti-neoplastic drug used solely or in combination with other anti-neoplastic drugs for the treatment of leukemia and other diseases. Relatively low doses of methotrexate (7.5 – 25 mg/week) have been used in the treatment of nonmalignant diseases such as severe psoriasis, asthma, rheumatoid arthritis, sarcoidosis, and transplantation therapy. Intermediate to high doses of methotrexate (35 $mg/m^2$ – 12 $g/m^2$) with leucovorin (citrovorum-factor) rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer. Patients undergoing methotrexate therapy should be closely monitored so that toxic effects are detected promptly.

Methotrexate serum levels depend on dosage, mode of administration, treatment regimen, individual pharmacokinetics, metabolism and other clinical factors. While the serum level may typically reach 10 to 100 µmol/L, 15 concentrations may exceed 1000 µmol/L with high dose therapy for osteosarcoma, and up to 3100 µmol/L methotrexate was reached following a 4-hour infusion in pediatric patients with osteosarcoma.

For treatment of osteosarcoma, the methotrexate decay curve has wide variability: 24 hours, 30 to 300 µmol/L; 48 hours, 3 to 30 µmol/L; and 72 hours, < 0.3 µmol/L. A dose of 10 mg of leucovorin is usually administered intravenously 24 hours after initiation of the MTX infusion. Subsequent doses are adjusted and administered according to the MTX levels obtained at 24, 48, and 72 hours. Methotrexate levels in excess of 50 µmol/L at 24 hours, 10 µmol/L at 48 hours, and 0.5 µmol/L at 72 hours portend potential toxicity and are usually treated with an increase in the dose of leucovorin. Guidelines for methotrexate therapy with leucovorin rescue usually recommend continuance of leucovorin until the methotrexate level falls below 0.05 µmol/L.

Renal toxicity is a significant risk and may be exacerbated by coadministration of other drugs, for example vancomycin. Other forms of toxicity can occur, including digestive disorders (e.g., nausea, vomiting, abdominal pain), cutaneous–mucous disorders (especially mucositis), haematological abnormalities (e.g., neutropenia and thrombocytopenia), liver function test disturbances, and neurotoxicity.

Given the profile of the appearance of the 7-hydroxymethotrexate metabolite, its molar ratio to methotrexate of up to approximately 100-fold, and relative insolubility versus the parent drug, possible nephrotoxicity due to precipitation of the metabolite in renal tubules may delay elimination of methotrexate itself. Glucarpidase therapy (available for compassionate use) reduces the circulating level of methotrexate rapidly, not the intracellular drug. A rebound effect in the serum level of methotrexate following glucarpidase therapy has been observed.

## Analyzer

Abbott Alinity ci or Abbott Alinity c system, Minneapolis Lab

## Sunquest Test Codes

MTX Methotrexate CPT: 80299

## Sample

**Sample:** Serum, no gel

**Draw volume: 2.5 mL blood**
**Preferred sample volume: 500** µL **Minimum volume:** 200 µL **Actual sample volume**: 5 µL

- Refer to specimen collection procedures for collection of diagnostic blood specimens
- Use the same specimen matrix for individual patients
- The sampling time of methotrexate is dependent on dose, duration of infusion, and clinical status of the patient. Consult specific Heme/Onc treatment protocols and Physicians' Desk Reference (PDR) for sampling time information
- Do not shake, create foam, or bubbles.
- Fibrin, red blood cells, and other particulate matter may cause an erroneous result. Ensure adequate centrifugation.

**Stability:**
2 to 8°C / two weeks
-20°C /3 freeze-thaw cycles

**Rejection criteria**: Unlabeled specimens, samples other than serum without gel.

**Preparation:**
1. Whole blood specimens should be centrifuged following complete clot formation according to Specimen Processing procedures prior to analysis
2. Serum should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Samples may still be run if unprotected from light for up to 5 hours.
4. Lipemic samples may be ultrafuged.
5. Specimens should be free of particulate matter.
6. Transfer serum to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

## Materials

| Supplies |
|---|
| • Empty Black Alinity cartridge<br>• Transfer pipettes<br>• Sample Cups, sendout tube with cap |

## Reagent Preparation

Reagents R1 and R2 need to be transferred to Vista-specific reagent containers prior to use. Avoid cross-contamination of R1 and R2

| Reagents | Stability | Preparation |
|---|---|---|
| **ARK Methotrexate Assay Reagent – Antibody/Substrate R1**<br><br>**5026-0001-00**<br>1 X 16 mL | **Unopened:** 2–8°C, upright and tightly closed, expiration date printed on the label<br><br>Do not freeze reagents. Avoid prolonged exposure to temperatures above 32°C<br><br>Improper storage of reagents can affect assay performance | Liquid, ready to use, may be used directly from the refrigerator<br><br>Reagents **R1** and **R2** are provided as a matched set and should not be interchanged with reagents from different lot numbers. |
| **Reagent – Enzyme R2**<br><br>**5026-0001-02**<br>1 X 8 mL | | |
| **ARK Methotrexate Calibrator**<br><br>**5023-0002-00** | **Unopened**: 2-8°C, date on vial<br><br>**Opened**: 2-8°C and tightly capped, 12 months or date on vial | Ready to use. Mix by gentle inversion before dispensing. |
| **ARK Methotrexate Calibration Range Controls**<br><br>**25026-0003-01** | **Unopened**: 2-8°C, date on vial<br><br>**Opened**: 2-8°C and tightly capped, 12 months or date on vial | Controls are ready to use. Mix each level by gentle inversion before dispensing. |
| **ARK Methotrexate Dilution Buffer**<br><br>5026-0004-00 | **Unopened**: 2-8°C, date on vial<br><br>**Opened**: 2-8°C and tightly capped, 12 months or date on vial | Dilution Buffer is ready to use. Mix by gentle inversion before dispensing.<br><br>Composition is equivalent to Calibrator A (zero) |

## Filling the Reagent Cartridge

| STEP | ACTION |
|---|---|
| 1. | Obtain an empty, black Alinity reagent cartridge and remove the caps. |
| 2. | Add Methotrexate Reagent 1 and Reagent 2 by following steps 3-4 |
| 3. | In the larger cartridge bottle, gently pour or use a transfer pipette to transfer the entire bottle of **ARK Methotrexate Assay Reagent R1 –Antibody/Substrate (16 mL)** |
| 4. | In the smaller cartridge bottle, gently pour or use a transfer pipette to transfer the entire bottle of **ARK Methotrexate Reagent R2–Enzyme (8 mL)** |
| 5. | Utilizing the Abbott Alinity Operating Manual, print a 1D barcode, ensuring the lot number of the reagent matches the barcode. |
| 6. | Affix the barcode to the cartridge as shown in the Operating Manual. Load the reagent on the RSM to initiate the analyzer automatic loading into the reagent wheel. |

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| 7. | **Run 3 levels of QC on each new reagent cartridge to verify viability of reagent.** |

## Calibration

Perform a full calibration (6-point) procedure using the ARK Methotrexate Calibrators A, B, C, D, E, and F in duplicate.

| | | |
|---|---|---|
| Assay Range: | 0.04 - 1.20 µmol/L. | |
| Reference Material: | ARK™ Methotrexate Calibrator<br>5026-0002-00 | |
| Suggested Calibration Levels: | A | 0.00 µmol/L |
| | B | 0.05 µmol/L |
| | C | 0.15 µmol/L |
| | D | 0.25 µmol/L |
| | E | 0.50 µmol/L |
| | F | 1.20 µmol/L |

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| Calibration Scheme: | Six levels in duplicate. Verify the calibration with 3 levels of QC<br>Add 200uL of calibrator into individual sample cups for each level. Return caps to their original containers and keep tight. |
| Calibration Frequency: | • Whenever a new lot number of reagents is used<br>• Whenever indicated by quality control results<br>• Whenever required by standard laboratory protocols<br>• Once every 14 days |
| Analytical Measuring Range | 0.04 - 1.20 µmol/L. The AMR is verified with each calibration using 6 levels of calibrator that span the full reportable range. Further studies are not necessary. |

## Add a New Lot of Calibrators

| STEP | ACTION |
|---|---|
| 1. | Log in as ADMIN (Password 8642. If possible, start with the c-side module in Idle. |
| 2. | Select **Configuration** from the drop down menu on the main screen |
| 3. | Select **Calibrator** |
| 4. | Select **Methotrexate** calibrator |
| 5. | Enter the lot number and expiration date. Make lot the default by checking the box. |
| 6. | Select **Save** and Done. Ensure you select the correct lot number when ordering calibration. |

## Quality Control

**ARK Methotrexate Control (2 mL)** vials
- LOW (0.07 µmol/L)
- MID (0.40 µmol/L)
- HIGH (0.80 µmol/L)
- Dilution Control 500 µmol/L

Use each lot as a set; do not mix lot numbers or bottles from separate kits

**Frequency:**
- Three levels once each day of patient testing.
- After loading a new reagent cartridge
- When quality control results or analyzer function warrant a quality check
- Dilution control only: This control is to be serial diluted each time a patient sample is diluted to verify dilution technique.

**Stability:**
- **Unopened**: 2°- 8°C. Use prior to expiration date on container
- **Open:** until expiration date on label when stored tightly capped at 2°- 8°C

**Acceptable ranges:**
- Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
- Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte.
- **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
- In the event of a QC failure, refer to the Unity Real Time QC Review, General User and navigate to the QC Troubleshooting section.
- Do not load or release patients until QC is acceptable in Unity Real Time.

**Interferences**

Interference studies were conducted using CLSI/NCCLS Protocol EP7-A2 as a guideline. Clinically high concentrations of the following potentially interfering endogenous substances in serum with known levels of methotrexate (approximately 0.05 and 0.50 µmol/L) were evaluated. Each sample was assayed using the ARK Methotrexate Assay, along with a serum control of methotrexate. Measurement of **methotrexate was not substantially affected** at the levels of endogenous substances tested (Albumin, Bilirubin – conjugated, Bilirubin – unconjugated, Cholesterol, Gamma-Globulin, Hemoglobin, Intralipid®, Rheumatoid Factor, Triglycerides, Uric Acid)

*Specificity*

*Cross reactivity to 7-Hydroxymethotrexate, the major metabolite*

- The ARK Methotrexate Assay did not cross-react (≤ 0.07%) with the major metabolite 7-hydroxymethotrexate.
- After administration of high-dose methotrexate (HDMTX), the serum/plasma concentration of 7-hydroxymethotrexate typically exceeds that of methotrexate at later time points. It has been reported that 7-hydroxymethotrexate levels exceed those of methotrexate by up to 100-fold 12 to 48 hours after HDMTX administration.

The clinical team should notify the laboratory when glucarpidase is administered to avoid the reporting of falsely elevated methotrexate concentrations due to interference by DAMPA.

*Drugs that cross-react*
The ARK Methotrexate Assay cross-reacts slightly with triamterene and trimethoprim. 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.

**Note:** glucarpidase (carboxypeptidase G2) is administered rarely at Children's in the event of renal failure. Pharmacy is aware of the cross-reactivity. Triamterene has not been available at Children's. Trimethoprim is contraindicated with methotrexate therapy, and is discontinued prior to methotrexate treatment.

**Reference Range**

None established.
Laboratory Indicators of Toxicity Following Leucovorin Rescue Schedules with High Dose Methotrexate.

| Clinical Situation | Laboratory Findings | |
|---|---|---|
| | Methotrexate Level (µmol/L) | Hours after administration |
| Normal Methotrexate Elimination | ~10 | 24 |
| | ~1 | 48 |
| | <0.2 | 72 |
| Delayed Late Methotrexate Elimination | >0.2 | 72 |
| | >0.05 | 96 |
| Delayed Early Methotrexate Elimination and/or Evidence of Acute Renal Injury | ≥50 | 24 |
| | ≥5 | 48 |
| | OR | |
| | ≥100% increase in serum creatinine | 24 |

**Critical Values**

>1.0 µmol/L Critical values must be called according to the Critical Limit Reporting Policy

**Limitations**

Analytical Measuring Range: 0.04 - 1.20 µmol/L.

The instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in open channel method results. Refer to your Dimension Vista 500® Operator's Guide for the meaning of report flags and comments. Any report containing flags and/or comments must be resolved prior to reporting.

## Dilutions

**Above 1.20 µmol/L**: Perform a manual dilution using the procedure outlined below.

| MTX on Serum | |
|---|---|
| Maximum Manual Dilution | 1:1000 |

| STEP | INSTRUCTIONS |
|---|---|
| 1. | Manually dilute the high specimen and the dilution control with **ARK Methotrexate Dilution Buffer** by preparing the appropriate ten-fold serial dilution as shown below in sendout tubes and/or Abbott sample cups. Ensure each dilution is properly labeled with patient name, DOB, date, initials, and dilution factor. |

| Sample | Volume | Dilution Buffer Volume | Dilution | Dilution Factor |
|---|---|---|---|---|
| Undiluted | 50 µL | 450 µL | 1:10 | 10 |
| 1:10 sample | 50 µL | 450 µL | 1:100 | 100 |
| 1:100 sample | 50 µL | 450 µL | 1:1000 | 1000 |

Refer to the procedure CH 2.03 Dilution Preparation.

The maximum dilution to prepare is a 1:1000

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|---|---|
| 2. | .Program all patient dilutions manually in the Abbott Alinity software using the Abbott Alinity Operating Manual. Do not use the original sample ID; instead, use this ordering scheme: SIDx10, SIDx100, SIDx1000 so that results will not autofile. For the control, only program the 1:1000 dilution. Run all patient samples and the dilution control. |
| 3. | Enter dilution control values into Unity Real Time. The dilution control values must pass to accept the patient dilution results. **Use the value from the lowest possible patient dilution that produces a result without flags.** |
| 4. | Record manual dilution on the dilution log, and result using the Result Reporting section below. Have your dilutions checked by a second tech PRIOR to reporting results. |

## Result Reporting

- Results between **0.04 - 1.20** µmol/L without error messages are released
- Results below **0.04** µmol/L: report as < **0.04** µmol/L instead of the numerical value
- Results >**1.20** µmol/L are reported as the numerical result following a maximum dilution of 1:1000
- Results that exceed the assay range following the maximum dilution are reported as >**1200.0** µmol/L
- To convert µmol/L to µg/mL, divide the value obtained by the conversion factor of 2.2005

## Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer.

## Alternate Method

- Perform testing on the Abbott Alinity c backup (MALIC) if the main analyzer, MALCI/MACC, is out of service. Reagents must be loaded, calibrated, and quality controlled prior to releasing results, as Methotrexate is not routinely loaded on this analyzer.
- As loading MALIC will take some time, notify the provider of the situation and that there will be a slight delay.

## References

1. ARK™ *Methotrexate* Assay package Insert**,** Revised August 2017, 1600-0213-00 Rev 07, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538 USA Tel: 1-877-869-2320, Fax: 1-510-270-6298, customersupport@ark-tdm.com, www.ark-tdm.com
2. ARK™ Methotrexate Calibrator Insert, Revised February 2017, 1600-0214-00 Rev 05, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538 USA Tel: 1-877-869-2320, Fax: 1-510-270-6298, customersupport@ark-tdm.com, www.ark-tdm.com
3. ARK™ Methotrexate Control Package Insert, 1600-0215-00 Rev 05 , Revised February 2017, ARK Diagnostics, Inc., 48089 Fremont Blvd, Fremont, CA 94538 USA Tel: 1-877-869-2320, Fax: 1-510-270-6298, customersupport@ark-tdm.com, www.ark-tdm.com
4. Jacobs & DeMott Laboratory Test Handbook, 5th Edition, Lexi-Comp, Inc., Hudson, OH, 2001
5. Abbott Alinity ci-series Operations Manual

## Historical Record

| Version | Written/Revised by: | Effective Date: | Summary of Revisions |
|---|---|---|---|
| 1. | Erin Bartos | 10/28/2020 | New method on Abbott Alinity |
| 2. | Matt Johnson | 2/14/2022 | Added Dilution control (500 µmol/L) to Controls and Dilution sections. |