Methotrexate by Immunoassay
Abbott Architect

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Introduction

Methotrexate [N-[4[[(2,4-diamino-6-pteridinyl) methyl] methylamino] benzoyl] –Lglutamic acid] is a folic acid antagonist that has been used as an antineoplastic agent since the 1950s and more recently has been used for the treatment of autoimmune disorders. The drug is available as a free acid or sodium salt in tablets for oral administration and in vials for parenteral injection. Dosage schedules vary, but often involve daily oral administration for up to eight days or once weekly administration by any route. High dose therapy, currently used against certain cancers, consists of rapid or slow intravenous infusion of up to 15 g/m^2 of the drug. This is followed by the frequent administration of leucovorin, an antidote that acts by restoring the body's pool of reduced folate to prevent damage to normal cells.[6] Toxic effects of methotrexate include, bone marrow suppression, mucositis and hepatic cirrhosis.

Principle

The ARK Methotrexate assay is a homogeneous enzyme immunoassay used for the quantitative determination of methotrexate in human serum or plasma.

Equipment

Abbott Architect c8000, c16000 (Abbott Diagnostics, Abbott Park IL)

Specimen Collection and Handling

Specimen requirements are 1 red top tube (minimum 2 ml of serum), protected from light. Samples should not be submitted in tubes that contain a serum separator. Samples should be stored at 2-8 ^{o}C prior to analysis.

Reagents

1. Methotrexate Assay Reagent (ARK Diagnostics) (5026-0001-00) , ready to use - R1(16 mL) - Antibody/Substrate contains rabbit polyclonal antibodies to methotrexate, glucose-6-phosphate, nicotinamide adenine dinucleotide, bovine serum albumin, preservative and stabilizers. R2(8 mL) – Enzyme contains

methotrexate labeled with bacterial G6PDH, buffer, bovine serum albumin, preservative and stabilizers. R1 and R2 must be used as a matched set and should not be interchanged with reagents from different lot numbers. Stable until expiration date if stored at 2-8ºC with screw caps tightly closed.

2. Methotrexate Calibrator (ARK Diagnostics) (5026-002-00) Six levels of methotrexate, 2 mL of each. Stable until expiration date when stored at 2-8ºC. Once opened, vials stored at 2-8 ºC are stable for 12 months.

3. Methotrexate Controls (ARK Diagnostics) (5026-0003-00) Six levels of methotrexate, 2 mL each . Stable until expiration date when stored at 2-8ºC. Once opened, vials stored at 2-8 ºC are stable for 12 months.

4. Methotrexate Dilution Buffer (ARK Diagnostics) (5026-0004-00) 25 mL. contains buffer, bovine serum albumin and preservatives. Stable until expiration date when stored at 2-8ºC. Once opened, stable when stored at 2-8 ºC for 12 months.

Quality Control

The LMH QC will be run daily by the Dayshift. Dilution Range controls (5,50,500) will be run once a week as part of weekly maintenance. Results should not be reported when QC limits are exceeded unless approved by supervisory staff.

Procedure

1. Verify the expiration date of the reagent. Do not use expired reagents.

2. Invert the reagent bottles gently to insure homogeneity.

3. Pour R1 and R2 reagents into properly labeled 20 mL user defined cartridges. Pour MTX diluent into a 50 mL user defined cartridge.

4. Remove air bubbles. (An applicator stick can be used for this purpose).

5. Methotrexate testing is performed on the Architect Chemistry Analyzer using user defined settings. System must be in ADMIN to open a new lot of reagent.

6. Select System at the top of the screen, Configuration, Assay Categories.

7. Select Reagent Settings, MTX, Configure (F-6)

8. The Configure reagent window displays.

9. Select the New Lot option under lot number. Enter the Lot Number, Expiration and Serial Number. (Note: The serial number is required. Use the date the reagent is put in use.)

10. Define the cartridge size 20mL. Select Done.

11. Packs of the same lot can be replaced in the same location as the onboard pack by selecting Reagents, Highlighting the Reagent Pack and Selecting F-8 Reset.

12. Samples are tested from the automation line or from the sample carousel.

13. Results will upload to Instrument Manager and the Laboratory Information System.

Entering New Calibrator Set Points

1. Instrument must be in "Ready" state and the operator needs to be signed in as "ADMIN".
2. Go to System, Configuration, QC-Cal Settings, Calibrator Set, Highlight (MTXCal), Configure.
3. Go to Define data. Enter the new values.
4. Go to System, Configure, Assay Settings, Retest Rules, Select assay.
5. Configure, Edit Rule, Result Range.
6. Enter the Cal 6 value.

For more detailed information, see the Abbott Architect Systems Operation Manual.

Standardization

Calibration is performed whenever a new lot number of reagent is used, whenever indicated by quality control results, when a new reagent pack is opened or every six months.

Safety Precautions

Universal precautions are indicated when handling patient specimens and quality control materials. Spills and accidents should be addressed immediately.

Analytical Range

The analytical measurement range for methotrexate is 0.05 – 1.2 µmol/L. Samples with results greater than 1.2, will automatically be diluted by the instrument with the Methotrexate Diluent. The instrument will automatically dilute x10. If result is >12.0 µmol/L then the instrument can be programed to do a x100 dilution. If the result from the instrument dilution is >120 µmol/L, then a manual 1:10 dilution must be made by adding 50 µL of sample to 450 µL of dilution buffer to a tube and MIX WELL. Program the instrument to perform a x100 dilution. THIS RESULT MUST BE MANUALLY MULTIPLIED X10. If the result from the manual and instrument dilution is >1200 µmol/L then a serial manual dilution must be made:

Serially dilute the sample to 1:100 dilution
Add 50 µL of sample to 450 µL of dilution buffer (1:10). Mix well.
Add 50 µL of **1:10 diluted sample** to 450 µL of dilution buffer (1:100). Mix well.
Program the instrument to perform a x100 dilution.

THIS RESULT MUST BE MANUALLY MULTIPLIED X100. If the result from the serial manual and instrument dilution is >12,000 µmol/L, then **>12,000 µmol/L will be reported.**

Sample	AMR
Undiluted	0.05 – 1.20
x10 automatic instrument dilution	1.21 – 12.00
x100 instrument dilution	12.01 – 120.00
x1000 (1:10 Manual Dilution and x100 instrument dilution)	120.01 – 1200.00
x10000 (x 100 manual dilution and x100 instrument dilution)*	1200.01 – 12000.00

*If the final dilution gives a result >12000, then **>12000 µmol/L** will be reported.

Calculations and Interpretations

Patient results are automatically uploaded to Instrument Manager. Results needing operator attention remain in Review until released by the operator. The operator programs instrument dilutions and the patient result is automatically calculated using the dilution factor. Manual dilutions must be programmed by the operator for the dilution factor to be applied. Both a manual dilution and an instrument dilution CANNOT be simultaneously applied on the Abbott Architect. If a manual dilution and instrument dilution are used, then the result MUST BE MULTIPLIED by the manual dilution factor.

x1000 manual and x10 instrument dilution - instrument result must be multiplied by 1000

See MTX worksheet.

Interfering Substances

Consult the Product Information sheets for specific information on interferences with endogenous substances and other drugs.

Reference Range

Concentrations after a single, high-dose bolus should be:

<10.0 µmol/L after 24 hours
<1.00 µmol/L after 48 hours
<0.10 µmol/L after 72 hours

Concentrations greater than this may indicate toxicity and warrant antidote intervention.

References

1. Abbott Architect System Operation Manual, Abbott Laboratories, Abbott Park, IL.
2. ARK Methotrexate Package Insert, ARK Diagnostics, Inc., Fremont, CA.
3. ARK Methotrexate Control Package Insert, ARK Diagnostics, Inc., Fremont, CA.
4. ARK Methotrexate Calibrator Package Insert, ARK Diagnostics, Inc., Fremont, CA.
5. ARK Methotrexate Dilution Buffer Package Insert, ARK Diagnostics, Inc., Fremont, CA
6. Baselt, R., Disposition of Toxic Drugs and Chemicals in Man. 8th Ed., 2008.

Authorized Reviewers

Section Medical or Technical Director

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Updated by: Robin Carey-Ballough	3/22/21	r02	Correct AMR from 0.5 to 0.05. Change QC frequency to Dayshift only.	
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