ARCHITECT Methotrexate

REF 2P49-27

en

Methotrexate
2P49
611-131_R02
B2P4Q0

Revised August 2018.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

ARCHITECT Methotrexate

INTENDED USE

The ARCHITECT Methotrexate assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of methotrexate in human serum and plasma on the ARCHITECT iSystem. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

SUMMARY AND EXPLANATION OF THE TEST

Methotrexate (MTX, amethopterin) is a folate antimetabolite. It is an analog of aminopterin, which is also derived from folic acid. The molecular structure of methotrexate differs from folic acid in that it has a hydroxyl group in place of the 4-amino group on the pteridine ring and there is no methyl group at the N position.

Methotrexate is an antineoplastic drug used solely or in combination with other antineoplastic drugs for the treatment of leukemia and other diseases.[1, 2] Relatively low doses of methotrexate have been used in the treatment of nonmalignant diseases such as severe psoriasis, asthma, rheumatoid arthritis, sarcoidosis, and transplantation therapy.[3-8] Intermediate to high doses of methotrexate with Leucovorin rescue have been used with favorable results in the treatment of osteogenic sarcoma, leukemia, non-Hodgkin's lymphoma, lung and breast cancer.[9-13]

Methotrexate levels are monitored to avoid excessive toxic effects of the drug and to determine when to intervene with counter-acting 'rescue' therapy. Adverse reactions include myelosuppression, stomatitis, nausea, vomiting, convulsions, liver and renal abnormalities, anemia, leukopenia, thrombocytopenia, osteoporosis, neurotoxicity, and leukoencephalopathy.[2] There are no current indications for monitoring low dose methotrexate therapies.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Methotrexate assay is a one-step immunoassay for the quantitative determination of methotrexate in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, anti-methotrexate coated paramagnetic microparticles, assay specific diluent, and methotrexate acridinium-labeled conjugate are combined to create a reaction mixture. The methotrexate present in the sample binds to the anti-methotrexate coated microparticles and to the methotrexate acridinium-labeled conjugate.
2. After washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
3. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is an inverse relationship between the amount of methotrexate in the sample and the RLUs detected by the ARCHITECT iSystem optics.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT Methotrexate 2P49

REF	2P49-27
Σ	100
MICROPARTICLES	1 x 6.6 mL
CONJUGATE	1 x 15.0 mL
ASSAY SPECIFIC DILUENT	1 x 5.9 mL

MICROPARTICLES Anti-methotrexate (mouse monoclonal) coated microparticles in MES buffer with non-protein stabilizers. Minimum concentration: 0.073% solids. Preservative: ProClin 300.

CONJUGATE Acridinium-labeled methotrexate conjugate in citrate buffer with non-protein stabilizers. Minimum concentration: 22 ng/mL. Preservative: ProClin 300.

ASSAY SPECIFIC DILUENT MES buffered saline diluent. Preservative: ProClin 300.

Other Reagents

MULTI-ASSAY MANUAL DILUENT 1 x 100 mL ARCHITECT Multi-Assay Manual Diluent, REF 7D82-50, containing phosphate buffered saline solution. Preservative: antimicrobial agent.

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[14-17]

The following warnings and precautions apply to: MICROPARTICLES

WARNING	Contains methylisothiazolones and morpholinoethanesulfonic acid, monohydrate*.
H317	May cause an allergic skin reaction.
H316*	Causes mild skin irritation.

Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

The following warnings and precautions apply to: CONJUGATE	
	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: ASSAY SPECIFIC DILUENT	
	
WARNING	Contains methylisothiazolones, sodium thiocyanate and morpholinoethanesulfonic acid, monohydrate*.
H317	May cause an allergic skin reaction.
H316*	Causes mild skin irritation.
EUH032	Contact with acids liberates very toxic gas.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.

Disposal	
P501	Dispose of contents / container in accordance with local regulations.

* Not applicable where regulation EC 1272/2008 (CLP) or OSHA Hazard Communication 29 CFR 1910.1200 (HCS) 2012 have been implemented.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.
  - Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	May be used immediately after removal from 2-8°C storage. Store in upright position.
On board	System temperature	30 days	Discard after 30 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT Methotrexate assay files must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

There are separate assay files that allow the user to order automated dilutions through the user interface.

- MTX UNDIL (undiluted protocol)
- MTX_20DF (1:20 dilution protocol)
- MTX_400DF (1:400 dilution protocol)
- MTX_8000DF (1:8000 dilution protocol)

The MTX UNDIL (assay number 662) assay file must be installed before the dilution protocol assay files MTX_20DF (assay number 663), MTX_400DF (assay number 664), MTX_8000DF (assay number 665) can be installed.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

Edit assay parameter "Result concentration units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default result unit	Conversion factor	Alternate result unit
µmol/L	0.45444	µg/mL

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	SST Serum collected in tubes coated with silicone and micronized silica as a clot activator.
Plasma	Dipotassium (K2) EDTA Sodium Heparin Lithium Heparin

- Other specimen collection tube types have not been tested with this assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- Human serum or plasma samples tested for methotrexate should be protected from light.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Human serum or plasma samples tested for methotrexate should be protected from light.
- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed
  - obvious microbial contamination
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use disposable pipettes or pipette tips.
  - Replace disposable pipettes or pipette tips between samples.
  - Replace pipette tips between serial dilution steps for samples and controls.
- Replace gloves if contamination is suspected.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous. If samples are not mixed thoroughly, inconsistent results may be obtained.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged at a Relative Centrifugal Force (RCF) ≥ 10,000 x g for 10 minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they require repeat testing, or
  - they were frozen and thawed.
- Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	Room temperature	≤ 1 day
	2-8°C	≤ 2 days
	-10°C or colder	> 2 days

- Human serum or plasma samples tested for methotrexate should be protected from light.
- Specimens may be stored on or off the clot, red blood cells
  - for up to 1 day at room temperature
  - for up to 2 days at 2-8°C
- If testing will be delayed more than 2 days, remove serum or plasma from the clot or red blood cells and store at -10°C or colder.
- Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- It is recommended that specimens be removed from the clot or red blood cells.
- Ship at 2-8°C (wet ice) or frozen (dry ice). Protect from light.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

2P49 ARCHITECT Methotrexate Reagent Kit

Materials Required but not Provided

- ARCHITECT Methotrexate Assay files obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 2P49-01 ARCHITECT Methotrexate Calibrators
- 2P49-10 ARCHITECT Methotrexate Controls or other commercially available controls
- 2P49-15 ARCHITECT Methotrexate Extended Range Controls
- 7D82-50 ARCHITECT Multi-Assay Manual Diluent
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Microcentrifuge tubes or equivalent
- Pipettes or pipette tips (optional) to deliver the specified volumes.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.

  Maximum number of replicates sampled from the same sample cup: 10
  - Priority:

    Sample volume for first test: 60 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - ≤ 3 hours on board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - > 3 hours on board: Replace with a fresh sample (patient specimens, controls, and calibrators).
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT Methotrexate Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 5 drops

    for each control: 5 drops
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Specimens with a methotrexate value exceeding 1.500 µmol/L (0.682 µg/mL) are flagged with the code "> 1.500 µmol/L" ("> 0.682 µg/mL") and may be diluted using either the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

The system performs a 1:20, 1:400, or 1:8000 dilution of the specimen and automatically calculates the concentration of the specimen before dilution and reports the result.

Test patient samples using the assay files per the table below.

To Order	Order Assay File
Patient Sample (1:20 dilution)	MTX_20DF
Patient Sample (1:400 dilution)	MTX_400DF
Patient Sample (1:8000 dilution)	MTX_8000DF

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Manual Dilution Procedure

- The suggested dilution for the ARCHITECT Methotrexate assay is 1:20. Up to 3 serial dilutions are made to the sample (1:20, 1:400, and 1:8000).
- **If a sample requires dilution, run one replicate of the Medium control and the diluted X, Y, and Z controls along with the diluted samples.**
- **Caution:** In order to prevent cross contamination, **do not remove dropper tips** from the ARCHITECT Methotrexate control bottles. **Use caution when handling control bottles and caps.**

  Replace pipette tips between samples, controls, and serial dilution steps.
- All samples in **bold** font below will be tested. Some tubes prepared are for intermediate steps in the serial dilution procedure and will not be tested.

Note: For all manual dilutions:

Diluent = ARCHITECT Multi-Assay Manual Diluent

DF = Dilution Factor

Serial Dilution of Specimen > 1.500 µmol/L (> 0.682 µg/mL)

1. Label 3 microcentrifuge tubes to be used for the dilution of the specimen:
   Tube #1 DF=20
   Tube #2 DF=400
   Tube #3 DF=8000
2. Add 950 µL Diluent to each of the 3 tubes.
3. Add 50 µL of the patient specimen to Tube #1.
   a. Vortex the sample for 10-30 seconds to mix.
   b. Tube **#1 DF=20** is ready for testing.
4. Remove 50 µL from Tube #1 (Step 3b) and add it to Tube #2.
   a. Vortex the sample for 10-30 seconds to mix.
   b. Tube **#2 DF=400** is ready for testing.
5. Remove 50 µL from Tube #2 (Step 4b) and add it to Tube #3.
   a. Vortex the sample for 10-30 seconds to mix.
   b. Tube **#3 DF=8000** is ready for testing.

   Serial Dilution of Controls X, Y, and Z
6. Label 6 microcentrifuge tubes to be used for the dilution of the controls:
   Tube X DF=20
   Tube Y1
   Tube Y2 DF=400
   Tube Z1
   Tube Z2
   Tube Z3 DF=8000
   Note: Only the tubes labeled with "DF" will be tested.
7. Add 950 µL Diluent to each of the 6 tubes.
8. Dispense 5 drops of Control X into a separate tube.
   a. Remove 50 µL of Control X from this tube and add it to Tube X DF=20.
   b. Vortex the sample for 10-30 seconds to mix.
   c. Tube **X DF=20** is ready for testing.
9. Dispense 5 drops of Control Y into a separate tube.
   a. Remove 50 µL of Control Y from this tube and add it to Tube Y1.
   b. Vortex the sample for 10-30 seconds to mix.
   c. Tube Y1 will not be tested.
10. Remove 50 µL from Tube Y1 (Step 9c) and add it to Tube Y2 DF=400.
    a. Vortex the sample for 10-30 seconds to mix.
    b. Tube **Y2 DF=400** is ready for testing.
11. Dispense 5 drops of Control Z into a separate tube.
    a. Remove 50 µL of Control Z from this tube and add it to Tube Z1.
    b. Vortex the sample for 10-30 seconds to mix.
    c. Tube Z1 will not be tested.
12. Remove 50 µL from Tube Z1 (Step 11c) and add it to Tube Z2.
    a. Vortex the sample for 10-30 seconds to mix.
    b. Tube Z2 will not be tested.
13. Remove 50 µL from Tube Z2 (Step 12b) and add it to Tube Z3 DF=8000.
    a. Vortex the sample for 10-30 seconds to mix.
    b. Tube **Z3 DF=8000** is ready for testing.

- Test the Medium Control, Control X (1:20 dilution), Control Y (1:400 dilution), and Control Z (1:8000 dilution).
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.
- A single replicate of each ARCHITECT Methotrexate Low, Medium, and High Control must be tested to evaluate the assay calibration. Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0.000 to 1.500 µmol/L (0.000 to 0.682 µg/mL).
- Once an ARCHITECT Methotrexate calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - A reagent kit with a new lot number is used or
  - Controls are out of range.
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

The recommended control requirement for the ARCHITECT Methotrexate assay is that a single sample of each Low, Medium, and High Control be tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.

It is recommended that Controls X, Y, and Z be diluted and tested with manually diluted samples.

For detailed dilution procedure instructions and quality control, refer to the **PROCEDURE, Specimen Dilution Procedures** section of this package insert.

Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated test results are invalid and the samples must be retested. Recalibration may be indicated.

If a diluted control is out of its specified range, the associated specimen dilution test results are invalid. Specimens and controls must be prepared again. Refer to the **PROCEDURE, Specimen Dilution Procedures** section of this package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT Methotrexate assay belongs to method group 6.

RESULTS

Calculation

The ARCHITECT Methotrexate assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration curve.

For information on alternate result units, refer to the **INSTRUMENT PROCEDURE, Alternate Result Units** section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in µmol/L which meets the limits of acceptable performance for both imprecision and bias for an undiluted sample.

For the studies described in this package insert, the range is 0.040 µmol/L (Limit of Quantitation - LoQ) to 1.500 µmol/L (0.018 to 0.682 µg/mL).

LIMITATIONS OF THE PROCEDURE

- Samples to be tested for methotrexate should be protected from light.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the methotrexate results are inconsistent with clinical evidence, additional testing is recommended.
- Specimens from patients who have received glucarpidase (carboxypeptidase G_2) as a high-dose methotrexate rescue therapy should not be tested with the ARCHITECT Methotrexate assay for at least 48 hours following the last dose of glucarpidase.[18] These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridinyl) methyl]-methylamino]-benzoic acid (DAMPA) that result from metabolism of methotrexate by glucarpidase.[19] DAMPA crossreacts with the methotrexate antibody used in this assay. Oncologists on the clinical team should notify the laboratory when glucarpidase is administered.[20]
- Aminopterin is derived from folic acid and crossreacts with the methotrexate antibody used in this assay.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT Methotrexate that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.[21]
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.[22]

EXPECTED VALUES

No precise relationship between methotrexate serum levels and antineoplastic efficacy has been established, although levels below approximately 0.02 µmol/L were seen as necessary for resumption of DNA synthesis.[23] The correlation between serum methotrexate drug concentration and duration of tumor cell exposure has been shown to predict methotrexate toxicity.

Following high-dose methotrexate treatment with Leucovorin rescue, patients with a 24-hour serum methotrexate concentration > 10 µmol/L, a 48-hour level > 1.0 µmol/L, and a 72-hour level > 0.1 µmol/L are at an increased risk of toxicity.[24]

Toxicity is typically present in the form of myelosuppression, stomatitis, nausea, vomiting, convulsions, and liver and renal abnormalities.[2] Anemia, leukopenia, thrombocytopenia, osteoporosis, skin and mucosal involvement with a fatal outcome have also been reported. Neurotoxicity and leukoencephalopathy are also reported as toxic effects involving methotrexate.[2]

SPECIFIC PERFORMANCE CHARACTERISTICS

Data in the **SPECIFIC PERFORMANCE CHARACTERISTICS** section were generated using the ARCHITECT i2000SR System.

Assay results obtained in individual laboratories may vary from data presented.

Precision

The ARCHITECT Methotrexate assay is designed to have within-laboratory (total) imprecision of ≤ 7.5% CV for samples with methotrexate concentrations between 0.040 µmol/L and ≤ 12.500 µmol/L and ≤ 10% CV for samples with methotrexate concentrations > 12.500 µmol/L.

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP05-A2.[25] The ARCHITECT Methotrexate Controls, Extended Range Controls, and 5 levels of human serum panels (Panels 1, 3, and 5-7) and 2 levels of human plasma panels (Panels 2 and 4) were assayed. All samples were tested in replicates of 2 at 2 separate times per day for 20 days. Additional replicates for Panels 6 and 7 were performed to assess precision at the measuring interval boundaries. The data are summarized in the following table.

		Mean	Within Run		Within-Laboratory (Total)	
Sample	n	(µmol/L)	SD	%CV	SD	%CV
Low Control	80	0.072	0.0023	3.2	0.0029	4.0
Medium Control	80	0.436	0.0134	3.1	0.0148	3.4
High Control	80	0.949	0.0401	4.2	0.0402	4.2
Control X	80	10.011	0.4060	4.1	0.4642	4.6
Control Y	80	55.645	1.9365	3.5	2.4197	4.4
Control Z	80	554.900	20.0998	3.6	39.0768	7.0
Panel 1	80	0.065	0.0026	4.1	0.0029	4.6
Panel 2	80	0.101	0.0044	4.4	0.0053	5.3
Panel 3	80	1.103	0.0475	4.3	0.0547	5.0
Panel 4	80	7.966	0.2683	3.4	0.3084	3.9
Panel 5	80	1679.100	60.8687	3.6	83.4626	5.0
Panel 6	160	0.047	0.0020	4.4	0.0025	5.4
Panel 7	160	1.347	0.0554	4.1	0.0590	4.4

Recovery

The ARCHITECT Methotrexate assay is designed to have a mean recovery of 100 ± 10% for samples with methotrexate concentrations from 0.040 µmol/L to 1.500 µmol/L.

A study was performed with 5 serum and 5 plasma samples that were spiked with known amounts of methotrexate to create samples across the measuring interval. The concentration of methotrexate was determined using the ARCHITECT Methotrexate assay and the resulting percent recovery was calculated. The mean percent recovery was 97% for serum samples and 98% for plasma samples.

Linearity

The ARCHITECT Methotrexate assay is designed to have a deviation from linearity within ± 10% for samples with methotrexate concentrations from 0.040 µmol/L to 1.500 µmol/L.

A study was performed based on guidance from the CLSI document EP06-A.[26] Serum and plasma dilution sets with methotrexate concentrations ranging from 0.010 µmol/L to 2.001 µmol/L were prepared gravimetrically and evaluated. The observed deviation from linearity was within ± 10% for samples from 0.040 µmol/L to 1.500 µmol/L.

An additional study was performed using serum and plasma samples with methotrexate concentrations ranging from 1.2 µmol/L to 2500 µmol/L. The samples were prepared gravimetrically and diluted using serial 1:20 dilutions into the measuring interval with ARCHITECT Multi-Assay Manual Diluent. Regression of assayed methotrexate concentrations was linear throughout the range.

Sensitivity

Limit of Quantitation (LoQ)

The ARCHITECT Methotrexate assay is designed to have a Limit of Quantitation (LoQ) of ≤ 0.040 µmol/L.

Based on guidance from the CLSI document EP17-A2,[27] a study was performed with 4 zero-level samples and 8 low-level methotrexate samples (targeted to approximately 0.010, 0.020, 0.030, 0.040, 0.050, 0.080, 0.100, and 0.200 µmol/L). These samples were tested in 6 separate runs over 4 days using 2 reagent lots and 2 instruments. The LoQ is defined as the lowest amount of analyte in a sample that can be quantitatively determined with a Percent Total Allowable Error < 25%. The LoQ for the ARCHITECT Methotrexate assay was 0.020 µmol/L.

Limit of Blank and Limit of Detection

In the same study, the Limit of Blank (LoB) and Limit of Detection (LoD) were determined. The LoB was 0.005 µmol/L and the LoD was 0.009 µmol/L.

Interference

Potentially Interfering Drugs

A study was performed based on guidance from the CLSI document EP07-A2.[28] Potentially interfering drugs were evaluated to determine whether methotrexate concentrations were affected when using the ARCHITECT Methotrexate assay. The drugs listed below were spiked into samples with methotrexate concentrations at the medical decision points of approximately 0.050 µmol/L and 1.000 µmol/L. The samples were assayed, and the methotrexate concentrations of the spiked samples were compared to reference samples. The ARCHITECT Methotrexate Assay did not crossreact with the major metabolite 7-Hydroxymethotrexate.

The drugs were tested at 1000 µmol/L except where noted. The data are summarized in the following table.

The compounds Aminopterin and DAMPA were also tested in serum at 1000 µmol/L in the absence of methotrexate and the cross-reactivity was 61% and 46%, respectively.

	% Cross-Reactivity [a] Methotrexate Concentration	
Potentially Interfering Drug	**0.050 µmol/L**	**1.000 µmol/L**
Adriamycin	0	0
Aminopterin [b] (5 µmol/L)	43	72
Cyclophosphamide (1500 µmol/L)	0	0
Cytosine	0	0
DAMPA [b] (5 µmol/L)	46	83
Dihydrofolic Acid	0	0
5-Fluorouracil (3000 µmol/L)	0	0
Folic Acid	0	0
Folinic Acid (Leucovorin)	0	0
7-Hydroxymethotrexate	0	0
6-Mercaptopurine	0	0
Methopterine	0	0
5-Methyltetrahydrofolic Acid	0	0
DL-6-Methyl-5,6,7,8-Tetrahydropterine	0	0
Prednisolone	0	0
Pyrimethamine	0	0
Sulfamethoxazole (1600 µmol/L)	0	0
Tetrahydrofolic Acid	0	0
Triamterene	0	0
Trimethoprim	0	0
Vinblastine	0	0
Vincristine	0	0

[a] % Cross-reactivity = $\frac{\text{Mean Test Concentration - Mean Control Concentration}}{\text{Therapeutic Concentration}} \times 100$

[b] The highest concentration that could be quantitated was 5 µmol/L.

Potentially Interfering Substances

A study was performed based on guidance from the CLSI document EP07-A2.[28] Potentially interfering substances were evaluated to determine whether methotrexate concentrations were affected when using the ARCHITECT Methotrexate assay. The substances listed below were spiked into samples with methotrexate concentrations at the medical decision points of approximately 0.050 µmol/L and 1.000 µmol/L. The samples were assayed and the methotrexate concentrations of the spiked samples were compared to reference samples. The data are summarized in the following table.

		% Difference [a] Methotrexate Concentration	
Potentially Interfering Substance	**Interferent Concentration**	**0.050 µmol/L**	**1.000 µmol/L**
Bilirubin (Unconjugated)	60 mg/dL	4	4
Bilirubin (Conjugated)	60 mg/dL	2	3
Cholesterol	700 mg/dL	4	3
Hemoglobin	1000 mg/dL	4	-3
Human Anti-Mouse Antibodies	1500 ng/mL	6	3
Monoclonal IgM	0.386 g/dL	7	-5
Rheumatoid Factor	1500 IU/mL	-4	8
Total Protein (albumin)	12 g/dL	-7	-6
Total Protein (gamma globulin)	12 g/dL	-5	-6
Triglycerides	3000 mg/dL	4	-3
Uric Acid	30 mg/dL	-4	2

[a] % Difference = $\frac{\text{Mean Test Concentration - Mean Control Concentration}}{\text{Mean Control Concentration}} \times 100$

Method Comparison

The ARCHITECT Methotrexate assay is designed to have a slope of 1.00 ± 0.10 and a correlation coefficient (r) of ≥ 0.95 for specimens ranging from 0.040 µmol/L to 1.000 µmol/L methotrexate when compared to the TDx method and ranging from 0.040 µmol/L to 1.500 µmol/L methotrexate when compared to the Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) method.

Studies were performed using serum specimens based on guidance from the CLSI document EP09-A3[29] using the Passing-Bablok regression method to compare the ARCHITECT Methotrexate assay to the TDx Methotrexate method and to the LC/MS/MS method. The data are summarized in the following tables including data from a study with specimens above the measuring interval requiring dilution.

ARCHITECT Methotrexate vs. TDx Methotrexate (N = 86)

Concentration Range (µmol/L)		Correlation Coefficient				
ARCHITECT	**TDx**	**(r)**	**Intercept**	**95% CI[a]**	**Slope**	**95% CI[a]**
0.040-0.993	0.05-0.99	0.9962	0.005	(0.001, 0.009)	0.946	(0.924, 0.970)

[a] CI = Confidence Interval

ARCHITECT Methotrexate vs. LC/MS/MS (N = 101)

Concentration Range (µmol/L)		Correlation Coefficient				
ARCHITECT	**LC/MS/MS**	**(r)**	**Intercept**	**95% CI[a]**	**Slope**	**95% CI[a]**
0.040-1.438	0.028-1.568	0.9960	0.016	(0.011, 0.022)	0.923	(0.906, 0.944)

[a] CI = Confidence Interval

ARCHITECT Methotrexate vs. TDx Methotrexate (N = 119)

Concentration Range (µmol/L)		Correlation Coefficient				
ARCHITECT	**TDx**	**(r)**	**Intercept**	**95% CI[a]**	**Slope**	**95% CI[a]**
0.040-888.000	0.05-855.50	0.9983	-0.004	(-0.007, -0.001)	1.016	(0.995, 1.037)

[a] CI = Confidence Interval

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date
ASSAY SPECIFIC DILUENT	Assay Specific Diluent
CONJUGATE	Conjugate
CONTAINS: CYANATE	Contains sodium thiocyanate. Contact with acids liberates very toxic gas.
CONTROL NO.	Control Number
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
MULTI-ASSAY MANUAL DILUENT	Multi-Assay Manual Diluent
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCED FOR ABBOTT BY	Produced for Abbott by
PRODUCT OF USA	Product of USA
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WASH BUFFER	Wash Buffer

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