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Methotrexate II

**REF** 7A12

34-5309/R7

**IVD**



# Methotrexate II

## Customer Service

United States: 1-877-4ABBOTT

International: Call your Abbott Representative

This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Read Highlighted Changes  
Revised February, 2009

### Key to symbols used

|  |  |
|--|--|
| <b>REF</b> List Number   | <b>LOT</b> Lot Number  |
| <b>IVD</b> <i>In Vitro</i> Diagnostic Medical Device                     | Expiration Date  |
| 2°C - 8°C Store at 2-8°C   | <b>CAL A</b> Calibrator (A-F)  |
| CAUTION: Consult accompanying documents                                  | <b>CONTROL L</b> Control Low, Medium, High, X, Y, Z (L, M, H, X, Y, Z) |
| <b>CUVETTES</b> Cuvettes   | <b>REAGENT PACK</b> Reagent Pack                                       |
| <b>DILUTION BUFFER</b> Dilution Buffer                                   | <b>SAMPLE CARTRIDGES</b> Sample Cartridges                             |
| Abbott Laboratories<br>Diagnostics Division<br>Abbott Park, IL 60064 USA | <b>EC REP</b> Authorized Representative                                |

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

This insert supersedes all previous inserts/manual inserts.

The contents of this insert have been reformatted. Changes other than reformatting are highlighted.

**COMMON NAME**

Methotrexate

**INTENDED USE**

The TDx/TDxFLx Methotrexate II assay is a reagent system for the quantitative measurement of methotrexate, an antineoplastic drug, in serum or plasma. The measurements obtained are used in monitoring levels of methotrexate to ensure appropriate therapy.

**SUMMARY AND EXPLANATION OF TEST**

The Methotrexate II assay utilizes Fluorescence Polarization Immunoassay (FPIA) technology. Refer to your TDx or TDxFLx System Operation Manual in the System Description section for a discussion of this technology.

Methotrexate is an antineoplastic drug used solely or in combination with other antineoplastic drugs for the treatment of leukemia and other diseases.<sup>1,2</sup> Relatively low doses of methotrexate (approximately 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.<sup>3-8</sup> Intermediate to high doses of methotrexate (approximately 35 mg/m<sup>2</sup> - 12 g/m<sup>2</sup>) 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.<sup>9-13</sup> The efficacy of methotrexate in the treatment of other tumors such as prostatic cancer is being investigated.<sup>14</sup>

**PRINCIPLES OF THE PROCEDURE**

Refer to your operation manual in the System Description section.

**REAGENTS**

| Item                                | Contents  |
|-------------------------------------|---|
| Reagent Pack (batch)                | <b>W</b> Wash Solution. Solvent. (4 mL).<br>Preservative: Sodium Azide.   |
|                                     | <b>S</b> <0.01% Methotrexate Antibody (Mouse Monoclonal) in buffer with protein stabilizer (4.0 mL).<br>Preservative: Sodium Azide.             |
|                                     | <b>T</b> <0.01% Methotrexate Fluorescein Tracer in buffer containing surfactant and protein stabilizer (3.5 mL).<br>Preservative: Sodium Azide. |
|                                     | <b>P</b> Pretreatment Solution. Surfactant in buffer containing protein stabilizer (3.5 mL).<br>Preservative: Sodium Azide.                     |
|                                     | <b>Methotrexate Concentration (µmol/L)</b>  |
| Calibrators (2.5 mL) in human serum | <b>CAL A</b> 0.00   |
|                                     | <b>CAL B</b> 0.05   |
|                                     | <b>CAL C</b> 0.15   |
|                                     | <b>CAL D</b> 0.30   |
|                                     | <b>CAL E</b> 0.60   |
|                                     | <b>CAL F</b> 1.00   |

Preservative: Sodium Azide.

Abbott manufactures internal reference standards for Methotrexate using Methotrexate Reference Standard (USP Grade). Methotrexate calibrators are manufactured gravimetrically and tested against these internal reference standards.

|                                  | Target Conc. (µmol/L)   | Range (µmol/L)  |
|----------------------------------|-------------------------|-----------------|
| Controls (2.5 mL) in human serum | <b>CONTROL L</b> 0.07   | 0.04 - 0.10     |
|                                  | <b>CONTROL M</b> 0.40   | 0.34 - 0.46     |
|                                  | <b>CONTROL H</b> 0.80   | 0.67 - 0.93     |
|                                  | <b>CONTROL X</b> 5.00   | 4.00 - 6.00     |
|                                  | <b>CONTROL Y</b> 50.00  | 37.20 - 62.80   |
|                                  | <b>CONTROL Z</b> 500.00 | 369.19 - 630.81 |

Preservative: Sodium Azide.

**Warnings or Precautions For Users**

For *In Vitro* Diagnostic Use.



**CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the **REAGENTS** section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens<sup>15</sup>. Biosafety Level 2<sup>36</sup> or other appropriate biosafety practices<sup>16,17</sup> should be used for materials that contain or are suspected of containing infectious agents.

The human serum used in the Methotrexate II Calibrators and Controls is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2.

This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

Refer to your operation manual in the System Description section.

**Storage Instructions**

 2°C - 8°C Store reagents, calibrators, and controls refrigerated at 2-8°C (35-46°F).

**Indications of Stability**

Reagents are stable until the expiration date when stored and handled as directed.

For additional information, refer to the **Calibration and Quality Control** sections in this insert.

**INSTRUMENT PROCEDURE**

The following instrument software is required to perform the assay:

For TDxFLx-Revision 3.0 or higher.

For TDx-Revision 16.0 or higher.

**Assay Parameters Methotrexate**

The Methotrexate II assay parameters are factory set for twenty-one (21) different functions. Refer to your operation manual in the Operation section for an explanation of assay parameters.

Before beginning the initial calibration and subsequent analysis with the Methotrexate II assay, press **ASSAY, 22**, and **PRINT** on the control panel. Verify the parameters with the following assay illustration.

|       |              |         |
|-------|--------------|---------|
| 22.   | METHOTREXATE |         |
| 22.1  | SPL VOL      | 10.0    |
| 22.2  | SPL REP      | 1       |
| 22.3  | LOLIM        | 0.00    |
| 22.4  | HILIM        | 1000.00 |
| 22.5  | CAL VOL      | 10.0    |
| 22.6  | CAL REP      | 2       |
| 22.7  | CONC A       | 0.00    |
| 22.8  | CONC B       | 0.05    |
| 22.9  | CONC C       | 0.15    |
| 22.10 | CONC D       | 0.30    |
| 22.11 | CONC E       | 0.60    |
| 22.12 | CONC F       | 1.00    |
| 22.13 | UNITS        | 2       |
| 22.14 | CRV FIT      | 2       |
| 22.15 | MX DEV       | 5.0     |
| 22.16 | MN POLA      | *       |
| 22.17 | MN SPAN      | #       |
| 22.18 | MODE         | 42      |
| 22.19 | GAIN         | *       |
| 22.20 | MX BKG       | 2500.00 |
| 22.21 | MN TR        | *       |

To edit a parameter, follow these steps:

1. Press **ASSAY**, **22**, . , plus the parameter number you wish to change, and **EDIT**.
2. Enter the new parameter value. Press **STORE**.
3. Press **NEXT** to continue to the next parameter. Press **STORE** after each parameter is edited.
4. After editing has been completed, press **STOP**.

The instrument will return to operational status and the display panel will read:

**READY**

- NOTES:**
- \* Parameters cannot be edited.
  - # Parameter can be edited and may vary with reagent pool changes.

Refer to the operation manual for a discussion of:

- Quality Control
- Installation Procedures
- Methods of Operation
- Performance Characteristics
- Assay Procedures
- Barcode Override
- Dilution Protocol
- Calibration Procedures
- Operational Precautions and Limitations
- Maintenance and Component Replacement

### SAMPLE COLLECTION AND PREPARATION FOR TESTING ANALYSIS

Serum and plasma (EDTA, heparin, potassium oxalate or sodium fluoride) specimens may be used with the Methotrexate II assay. Methotrexate specimens should be protected from light.<sup>18</sup> For further information, refer to your operation manual in the System Description section.

The sampling time of methotrexate will be dependent on dose, duration of infusion, and clinical status of the patient. Consult specific treatment protocols and Physicians' Desk Reference® (PDR) for sampling time.

### Interferences

The Methotrexate II assay is designed to deliver less than or equal to 10% interference for bilirubin, cholesterol, hemoglobin, total protein, and triglycerides. Typical results from interference studies are presented in the table below.

| Potential Interferent | Interferent Conc | Interference |
|-----------------------|------------------|--------------|
| Bilirubin             | 20 mg/dL         | ≤10%         |
| Cholesterol           | 700 mg/dL        | ≤ 5%         |
| Hemoglobin            | 0.86 g/dL        | ≤ 5%         |
| Total Protein         | 8 g/dL           | ≤ 5%         |
| Triglycerides         | 1000 mg/dL       | ≤ 5%         |

### METHOTREXATE II PROCEDURE

The Methotrexate II assay has three different pipetting protocols. Further information is described in each protocol procedure section.

| MTX Protocol                  | Mode | Dilution Options     | Sample Volume           | Use                   |
|-------------------------------|------|----------------------|-------------------------|-----------------------|
| Dilution Protocol             | 42   | All Dilutes          | 80 µL                   | MTX conc. unknown     |
| Interactive Dilution Protocol | 42   | 1:1<br>1:10<br>1:100 | 80 µL<br>50 µL<br>50 µL | MTX conc. known       |
| MTX Assay                     | 43   | N/A                  | 50 µL                   | MTX conc. <1.0 µmol/L |

N/A = Not applicable

The range of the Methotrexate II calibration curve is 0.00-1.00 µmol/L. Samples containing methotrexate in higher concentrations are assayed by instrument dilution of the sample into the range of the calibration curve.

1. Calibration of the Methotrexate II assay is performed as described for other assays in your operation manual in the Operation section.

**NOTE:** Although Methotrexate II assay parameter 22.18 (MODE) is ordinarily set for Mode 42, the Methotrexate II calibration is performed as a Mode 43 assay. When the Methotrexate II calibration protocol is initiated through the barcode on the calibration carousel or through barcode override, the mode will automatically change to Mode 43 for the calibration and back to Mode 42 when calibration is completed. The operator need not edit MODE in assay parameters to perform a calibration.

2. Methotrexate II Controls L, M and H should be run with each calibration run and as required in Mode 43 sample runs. Methotrexate II Controls M, X, Y and Z are for the dilution protocols (Mode 42).

The Methotrexate II assay has the capability of determining methotrexate concentrations up to 1000 µmol/L in patient samples without manual dilution of the samples. In order to accommodate the wide range of methotrexate concentrations while maintaining adequate sensitivity, three protocols for running the Methotrexate II assay are provided.

Minimum sample volume required:

|       |         |  |
|-------|---------|--|
| 50 µL | Mode 43 | (0 - 1.0 µmol/L)                                     |
|       | Mode 42 | Interactive Dilution Protocol<br>1:10, 1:100, 1:1000 |
| 80 µL | Mode 42 | Interactive Dilution Protocol 1:1                    |
|       | Mode 42 | Dilution Protocol (MTX All Dilutes)                  |

**Methotrexate II Dilution Protocol**

This protocol is used for the patient sample whose methotrexate concentration range is unknown. The assay is performed with Mode 42 pipetting.

1. Select an Assay Carousel.
2. Place four sample cartridges for *each* patient sample to be assayed in the Assay Carousel.
3. Place a cuvette in every carousel position that contains a sample cartridge. Lock carousel.
4. Pipette a minimum of 80 µL of undiluted sample or control into the sample well of the first of the four sample cartridges. Samples and controls may be placed *only* in positions 1, 5, 9, 13 and 17 of the carousel.
5. The Methotrexate II Control Z should be run with each Methotrexate Dilution Protocol.
6. Place the Methotrexate II Reagent Pack into the analyzer.
7. Place the loaded Assay Carousel on the spindle. Close the door.
8. Press **RUN**.

**METHOTREXATE**

then

**MTX DIL PROTOCOL**

will be displayed.

9. The instrument will make 1:10, 1:100, and 1:1000 serial dilutions of the undiluted sample or control into the sample wells of the next three sample cartridges. All patient samples will be diluted in like manner. All the sample dilutions will then be pipetted into cuvettes, reagents added and background intensities and net polarizations determined. The instrument will identify the first dilution of each sample that gives a net polarization that lies within the calibration curve. The calculated concentration (corrected for dilution) will be printed out. The concentration printed out in µmol/L is the concentration of the original undiluted sample or control. A typical printout of sample results follows:

| MTX DILUTION PROTOCOL        |                 |        |         |
|------------------------------|-----------------|--------|---------|
| LOC                          | SAMPLES<br>CONC | NET P  | BLK I   |
| 1                            | 0.41            | 131.61 | 1565.44 |
| 2                            |                 | 198.58 | 1345.23 |
| 3                            |                 | 206.33 | 1317.02 |
| 4                            |                 | 205.67 | 1364.18 |
| ****CONCENTRATION = 0.41**** |                 |        |         |
| 5                            | 0.79            | 80.20  | 1606.90 |
| 6                            |                 | 190.21 | 1365.22 |
| 7                            |                 | 203.98 | 1320.76 |
| 8                            |                 | 205.17 | 1420.02 |
| ****CONCENTRATION = 0.79**** |                 |        |         |
| 9                            |                 | 31.76  | 1648.31 |
| 10                           | 4.83            | 120.01 | 1391.27 |
| 11                           |                 | 194.24 | 1324.50 |
| 12                           |                 | 203.98 | 1303.91 |
| ****CONCENTRATION = 4.83**** |                 |        |         |

**NOTE:** Results obtained using the Methotrexate II Dilution Protocol can be printed out *only* in units of µmol/L. If units are changed according to the procedure outlined in your operations manual in the Operation section, "NO VALID ANSWER" will be printed out instead of a concentration.

10. If the concentration of a sample falls between dilution ranges, or none of the net polarizations for a sample are within the calibration curve, "NO VALID ANSWER" will be printed out instead of a concentration. This happens under the following circumstances:
  - a) The 1:1 dilution concentration is greater than 1.0 µmol/L and the 1:10 dilution concentration is less than 0.9 µmol/L.
  - b) The 1:10 dilution concentration is greater than 10.0 µmol/L and the 1:100 dilution concentration is less than 9.0 µmol/L.
  - c) The 1:100 dilution concentration is greater than 100 µmol/L and the 1:1000 dilution concentration is less than 90.0 µmol/L.
  - d) The 1:1000 dilution concentration is greater than 1000 µmol/L.

In these cases, the sample should be diluted manually with XSYSTEMS Dilution Buffer and rerun in the Methotrexate II Dilution Protocol.

**Interactive Dilution Protocol**

This protocol is a modification of the Methotrexate II Dilution Protocol and is used when the methotrexate concentration range is known. The concentration ranges appropriate for each dilution are:

| MTX Conc. Range<br>(µmol/L) | Dilution        | Sample Volume |
|-----------------------------|-----------------|---------------|
| 0.0 - 1                     | 1:1 (undiluted) | 80 µL         |
| 0.9 - 10                    | 1:10            | 50 µL         |
| 9.0 - 100                   | 1:100           | 50 µL         |
| 90.0 - 1000                 | 1:1000          | 50 µL         |

The protocol is performed with Mode 42 pipetting. It is initiated through the barcode override sequence.

1. Select an Assay Carousel.
2. Place four sample cartridges for *each* patient sample to be assayed in the Assay Carousel.
3. Place a cuvette in every carousel position that contains a sample cartridge. Lock carousel.
4. Pipette undiluted sample or control into the sample well of the first of the four sample cartridges. Refer to the above table for sample volume. Samples and controls may be placed *only* in positions 1, 5, 9, 13 and 17 of the carousel.
5. The appropriate Methotrexate II Control (M, X, Y or Z) should be run with each Methotrexate II Interactive Dilution Protocol. The M Control should be run with the 1:1 dilution, the X Control with the 1:10 dilution, the Y Control with the 1:100 dilution, and the Z Control with the 1:1000 dilution.
6. Place the Methotrexate II Reagent Pack into the analyzer.
7. Place the loaded Assay Carousel on the center post. Close the door.

8. Initiate the Interactive Dilution Protocol through barcode override:

a) Press **ASSAY, 22, RUN**.

Only on TDxFLx,

# \_\_\_\_\_

will be displayed. Enter or scan the reagent 13-digit code and press **STORE**.

Further prompts are identical to TDx.

**CALIBRATION ?**

will be displayed.

b) Press **NEXT**.

**CAROUSEL # ?**

will be displayed.

c) Enter carousel # and press **STORE**.

d) The instrument will now display the dilutions in sequence. Press **NEXT** to display the next dilution.

**MTX DIL 1:1?**

**MTX DIL 1:10?**

**MTX DIL 1:100?**

**MTX DIL 1:1000?**

e) Press **STORE** after the appropriate dilution is displayed to initiate the assay. Only *one* dilution can be selected for all the samples in the carousel.

9. All samples will be diluted as described in the Methotrexate II Dilution Protocol, but only the dilution that was selected will be pipetted into the cuvettes. Reagents are added, background intensities and net polarizations are determined. A concentration corresponding to the dilution selected is printed, which is the concentration in  $\mu\text{mol/L}$  of the original undiluted sample.

Results from the Methotrexate II Dilution Protocol and the Interactive Dilution Protocol are printed out in groups of four. The first patient sample is shown on the printout in positions 1 - 4 followed by the summary concentration result. The second patient sample is shown in positions 5 - 8 followed by the summary concentration result. Sample three is in positions 9 - 12, sample four in positions 13 - 16 and sample five is shown in positions 17 - 20, each followed by the concentration result for that sample. A typical printout of sample results follows:

| MTX DILUTION PROTOCOL |                               |        |         |
|-----------------------|-------------------------------|--------|---------|
| LOC                   | SAMPLES<br>CONC               | NET P  | BLK I   |
| 1                     |                               |        |         |
| 2                     |                               |        |         |
| 3                     | 49.77                         | 117.83 | 1310.59 |
| 4                     |                               |        |         |
|                       | ****CONCENTRATION = 49.77**** |        |         |
| 5                     |                               |        |         |
| 6                     |                               |        |         |
| 7                     | 48.26                         | 120.02 | 1288.48 |
| 8                     |                               |        |         |
|                       | ****CONCENTRATION = 48.26**** |        |         |

**NOTE:** Results obtained using the Methotrexate II Interactive Dilution Protocol can be printed out *only* in units of  $\mu\text{mol/L}$ . If units are changed according to the procedure outlined in the operation manual in the Operation section, "NO VALID ANSWER" will be printed out instead of a concentration.

10. If **NEXT** is pressed after

**MTX DIL 1:1000?**

is displayed,

**MTX ALL DILUTES ?**

will be displayed.

Pressing **STORE** will initiate the Methotrexate II Dilution Protocol where all dilutions are assayed. This would be the procedure if it were necessary to do barcode override while performing the Methotrexate II Dilution Protocol.

11. It is possible that the net polarization for a dilution will lie within the calibration curve, but the concentration for that dilution will not meet the concentration range specifications listed for the Interactive Dilution Protocol.

If this occurs, "NO VALID ANSWER" will be printed out instead of a concentration. In this case, the sample should be rerun in the Methotrexate II Dilution Protocol.

### Methotrexate II Assay in Mode 43

This protocol is used only for patient samples whose methotrexate concentration is known to be less than  $1.0 \mu\text{mol/L}$ . The assay is performed in Mode 43. No dilution of these samples is necessary, since these can be read directly from the calibration curve.

1. Prepare an Assay Carousel as described in your operation manual in the Operation section. Samples or controls may be placed in consecutive positions on the carousel.
2. Edit the Methotrexate II assay parameter 22.18 MODE to 43.
  - a) Press **ASSAY, 22.18, EDIT, 43, STORE**.
  - b) Press **STOP**.
3. Place the Methotrexate II Reagent Pack into the analyzer.
4. Place the loaded Assay Carousel on the center post. Close the door.
5. Press **RUN** to initiate the assay.

6. At the completion of the run, the net polarization and calculated concentration of each sample is printed.
7. Edit assay parameter 22.18 back to Mode 42 if the Methotrexate II Dilution Protocol or the Interactive Dilution Protocol is desired.
8. Mode 43 should not be used as an initial screen of methotrexate levels with high and low samples in the same carousel. Although carryover in the assay is very low (<0.05%), there is the potential for significant carryover from a very high to a very low sample. If Mode 43 is used in this manner, values obtained on the screen should be verified by reassaying.

#### Materials Provided

7A12-69 TDx/TDxFLx Methotrexate II Assay System (100 Tests) includes:

**REAGENT PACK**

XSYSTEMS **CUVETTES** and

XSYSTEMS **SAMPLE CARTRIDGES**

#### Materials Not Provided

TDx or TDxFLx analyzer

Methotrexate II Calibrators, 7A12-01

Methotrexate II Controls, 7A12-10

XSYSTEMS **DILUTION BUFFER**, 9519

Pipettes

#### Calibration

An acceptable Methotrexate II assay calibration curve should meet the following criteria:

- a) Polarization Error (PERR) -4.00 to +4.00 for all calibrators.
- b) Root Mean Squared Error (RMSE) less than or equal to 3.00.
- c) All controls are within the acceptable ranges.

#### When to Recalibrate

Recalibration is required when:

- The memory circuit board (Board #2) is replaced.
- An assay activation (new reagent pool) is issued.

Recalibration *may* be necessary when:

- Assay control values fall outside of the acceptable range specified in the **REAGENTS** section in this insert.
- PERR or RMSE values are not acceptable as specified above.
- A new lot number of reagent is used.
- A new lot number of buffer is used.
- Any dispense component is replaced.
- Any instrument calibration procedure is performed.

To determine whether recalibration is required, this reagent system should be checked by assaying all the controls. If the control results are within range, patient samples may be run without need for recalibration. If the control results are not within range, refer to your operation manual in the Troubleshooting section.

#### Quality Control

The recommended control requirement is one Methotrexate Control level tested once every 8 hour shift, no less than two different controls per 24 hours which span the medical decision range. Controls should be run as patient samples and may be placed in any position on the Sample Carousel. If the quality control procedures in your laboratory require more control testing to verify test results, follow those procedures.

Ensure that assay control values are within the concentration ranges specified in the **REAGENTS** section of this insert.

At the discretion of the laboratory, selected quality control rules may be applied to the quality control data. As an example, a Levey-Jennings plot may be used to track the assay's performance. Refer to your laboratory Standard Operating Procedure and/or Quality Assurance Plan for additional details.

When an assay control does not meet the established criteria for acceptability, associated test results should be considered suspect. Your laboratory should evaluate results and take remedial corrective action prior to reporting test results. In conjunction with your laboratory's Quality Assurance Plan, refer to your operation manual in the Troubleshooting section.

#### RESULTS

The TDx and TDxFLx software calculates a best-fit curve equation that is used to generate a calibration curve. This curve is stored in memory and concentrations of drug in unknown samples are calculated from this curve using polarization values generated for each sample in the assay. For additional details in reporting results refer to your operation manual in the Operation section for printouts.

To convert  $\mu\text{g/mL}$  to  $\mu\text{mol/L}$ , multiply the value obtained by the conversion factor of 2.2005.

#### LIMITATIONS OF THE PROCEDURE

As with all analyte determinations, the Methotrexate II value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). HAMA present in serum may interfere in immunoassays which utilize mouse monoclonal antibodies.<sup>19,20</sup> *These specimens should not be assayed with the TDx/TDxFLx Methotrexate II assay.*

Specimens from patients who have received carboxypeptidase G2 as a high dose methotrexate rescue therapy should not be tested with the TDx/TDxFLx Methotrexate II assay. These specimens have increased serum levels of 4-[[2,4-diamino-6-(pteridiny)methyl]-methylamino]-benzoic acid (DAMPA) that cross-reacts with the methotrexate antibody used in this assay.<sup>21-23</sup>

The concentration range of the XSYSTEMS Methotrexate II assay (0.00 - 1000  $\mu\text{mol/L}$ ) is adequate to accurately determine the methotrexate concentration in most of the patient samples encountered. Occasionally samples contain methotrexate in concentrations greater than the highest limit (1000  $\mu\text{mol/L}$ ). These samples should be diluted manually with XSYSTEMS Dilution Buffer and rerun in the Methotrexate II Dilution Protocol.

For the infrequent serum or plasma sample with a background intensity greater than the MX BKG value, the BLK I reading will be printed followed by "HI". Refer to your operation manual in the Printed Error Codes section for additional information.

#### EXPECTED VALUES

No precise relationship between methotrexate serum levels and antineoplastic efficacy has been established, although levels below approximately 0.02  $\mu\text{mol/L}$  were seen as necessary for resumption of DNA synthesis.<sup>24</sup> The correlation between serum methotrexate drug concentration and duration of tumor cell exposure in predicting methotrexate toxicity has been demonstrated. Following a 4-6 hour intravenous methotrexate infusion with dosages ranging from 50  $\text{mg/m}^2$  - 15  $\text{g/m}^2$ , a patient with a 24-hour serum concentration of greater than 5 - 10  $\mu\text{mol/L}$ , a 48-hour level greater than 0.5 - 1.0  $\mu\text{mol/L}$ , and a 72-hour level greater than 0.2  $\mu\text{mol/L}$  is at an increased risk of toxicity if conventional low-dose leucovorin rescue is given. In this case, high dose leucovorin rescue would be indicated.<sup>25-27</sup> Toxicity is typically present in the form of myelosuppression, stomatitis, nausea, vomiting, convulsions, and liver and renal abnormalities.<sup>28</sup> Anemia, leukopenia, thrombocytopenia, osteoporosis, skin and mucosal involvement with a fatal outcome have also been reported.<sup>29-31</sup> Neurotoxicity and leukoencephalopathy are also reported as toxic effects involving methotrexate.<sup>32-34</sup>

#### SPECIFIC PERFORMANCE CHARACTERISTICS

##### Specificity

Cross-reactivity was tested for 7-hydroxymethotrexate, the major metabolite of methotrexate. The cross-reactivity was determined as the concentration difference between samples containing both the methotrexate with the cross-reactant versus samples containing only the methotrexate. At a metabolite concentration of 1000  $\mu\text{mol/L}$ , which corresponds to high plasma levels in the high dose methotrexate patients, a change in the apparent concentration of methotrexate was not detected.

Cross-reactivity was tested for DAMPA, which is normally a metabolite of methotrexate. The cross-reactivity was determined as the percent concentration difference between samples containing both methotrexate and metabolite and samples containing only methotrexate. At a methotrexate concentration of 5 µmol/L and a metabolite concentration of 5 µmol/L (seen in patients shortly after receiving carboxypeptidase G2 therapy), an increase of 26% in measured concentration of methotrexate was observed. In addition, in samples containing 1000 µmol/L of the metabolite without methotrexate, cross-reactivity can be as high as 59%.

The following compounds showed less than 1% cross-reactivity when tested up to 1000 µmol/L with the Methotrexate II assay.

|                                       |                  |
|---------------------------------------|------------------|
| Adriamycin                            | 5-Fluorouracil   |
| Cyclophosphamide                      | 6-Mercaptopurine |
| Cytosine                              | Methopterin      |
| Dihydrofolic Acid                     | Prednisolone     |
| DL-L-Tetrahydrofolic Acid             | Pyrimethamine    |
| DL-N-5-Methyl-Tetrahydro-folic Acid   | Sulfamethoxazole |
| DL-6-Methyl-5,6,7,8-Tetrahydropterine | Triamterene      |
| Folic Acid                            | Trimethoprim     |
| Folinic Acid (Leucovorin)             | Vinblastine      |
|                                       | Vincristine      |

### Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence and was determined to be 0.02 µmol/L.

### Precision

Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) protocol EP5-T2<sup>35</sup> using human serum with 0.07, 0.40, 0.80, 5, 50 and 500 µmol/L of methotrexate added. Results from these studies typically yielded CV's of less than 10% for the M, H, X, Y and Z controls and less than 15% for the L control. Representative data from these studies follow.

| Target value<br>(n= 80) | Concentration (µmol/L) |       |        |
|-------------------------|------------------------|-------|--------|
|                         | 0.07                   | 0.40  | 0.80   |
| Mean                    | 0.07                   | 0.40  | 0.83   |
| SD Within Run           | 0.005                  | 0.006 | 0.012  |
| CV Within Run (%)       | 7.9                    | 1.5   | 1.5    |
| SD Between Day          | 0.007                  | 0.018 | 0.042  |
| CV Between Day (%)      | 10.3                   | 4.5   | 5.1    |
| SD Total                | 0.009                  | 0.022 | 0.049  |
| CV Total (%)            | 14.0                   | 5.4   | 5.9    |
| Target value<br>(n= 80) | 5                      | 50    | 500    |
| Mean                    | 5.04                   | 49.81 | 495.16 |
| SD Within Run           | 0.17                   | 1.12  | 12.97  |
| CV Within Run (%)       | 3.4                    | 2.3   | 2.6    |
| SD Between Day          | 0.22                   | 1.57  | 19.04  |
| CV Between Day (%)      | 4.4                    | 3.2   | 3.8    |
| SD Total                | 0.28                   | 2.92  | 27.02  |
| CV Total (%)            | 5.6                    | 5.9   | 5.5    |

### Accuracy by Recovery

Recovery was determined by adding methotrexate to human serum and to buffer at concentrations of 0.15, 0.30 and 0.60 µmol/L. An analyzer was calibrated with serum calibrators and both the buffer samples and the serum samples were read as unknowns in replicates of five (5) relative to this calibration. % Recovery = ("serum concentration" divided by "buffer concentration") x 100.

| Added Concentration (µmol/L) | Concentration in Serum (µmol/L) | Concentration in Buffer (µmol/L) | Percent (%) Recovery |
|------------------------------|---------------------------------|----------------------------------|----------------------|
| 0.15                         | 0.14                            | 0.14                             | 100.00               |
| 0.30                         | 0.29                            | 0.28                             | 103.57               |
| 0.60                         | 0.55                            | 0.57                             | 96.49                |

### Accuracy by Correlation with Reference Assays

The Methotrexate II assay was compared to the Methotrexate assay and HPLC by assaying clinical samples of patients on methotrexate therapy. The data covers the entire dynamic range from 0.02 - 1000 µmol/L and was run using the regular (mode 43) and autodilution (mode 42) protocols on the TDxFLx for the Methotrexate II assay and modes 11 and 3 on the TDx for the Methotrexate assay. Representative data follow.

| TDxFLx Methotrexate II vs.        | Number of Observations | Intercept | Slope | Correlation Coefficient |
|-----------------------------------|------------------------|-----------|-------|-------------------------|
| MTX (TDx)<br>(0.02 - 1.0 µmol/L)  | 49                     | -0.02     | 0.93  | 0.989                   |
| MTX (TDx)<br>(0.02 - 1000 µmol/L) | 99                     | -0.24     | 0.95  | 1.00                    |
| HPLC<br>(0.03 - 1000 µmol/L)      | 95                     | -0.02     | 1.01  | 0.999                   |

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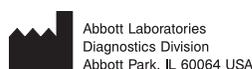
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