SIEMENS

Syva®

Emit® Methotrexate Assay

See shaded sections:
Updated information from 2017-06 edition.

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Methotrexate Assay

1 Intended Use

The Emit® Methotrexate Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of methotrexate in human serum or plasma.

2 Summary and Explanation of the Test

The use of leucovorin "rescue" permits relatively safe administration of very high doses of methotrexate to achieve maximum antineoplastic activity. Since the degree of methotrexate cytotoxicity is related to the drug's concentration and to the duration of exposure, leucovorin rescue dosages must be high enough and last long enough to avoid methotrexate toxicity. Monitoring methotrexate concentrations and rate of decline in serum during high-dose therapy is essential when designing adequate leucovorin rescue dosages for several reasons:[1]

- High extracellular concentrations of methotrexate may competitively inhibit the intracellular transport of leucovorin, thereby reducing leucovorin's effectiveness as a rescue agent.
- When methotrexate clearance is delayed by renal disease, ascites, pleural effusions, gastrointestinal obstruction, urinary pH, or other drugs, toxic concentrations may last beyond the usual duration of leucovorin rescue. Leucovorin dosage can be changed in such cases, but it must be done promptly; methotrexate toxicity may not be readily reversible if adequate rescue is delayed more than 42–48 hours.
- In patients with adequate methotrexate clearance, leucovorin dosage must not be so high as to reduce the drug's effectiveness against tumors.

The methods historically used to monitor serum methotrexate concentrations include radioimmunoassay (RIA), competitive reductase binding or radioenzymatic assay (REA), enzyme inhibition assay (DHFR), and high-performance liquid chromatography (HPLC) with either ultraviolet or fluorescence detection.[1]

3 Principle

The Emit® assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.[2] The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotometrically. Endogenous serum G6PDH does not interfere because the coenzyme functions only with the bacterial *(Leuconostoc mesenteroides)* enzyme employed in the assay.

4 Reagents

REF	Product Description	Quantity/Volume
6L119UL	**Emit® Methotrexate Assay** **Antibody/Substrate Reagent A** sheep antibodies reactive to methotrexate (88 µg/mL),† glucose-6-phosphate (66 mM), nicotinamide adenine dinucleotide (40 mM), Tris buffer, bulking agents, stabilizers, and preservatives	3 mL*
	Enzyme Reagent B methotrexate labeled with bacterial glucose-6-phosphate dehydrogenase (0.22 U/mL),† Tris buffer, bulking agents, stabilizers, and preservatives	3 mL*
	Emit® Drug Assay Buffer Concentrate when diluted, contains Tris buffer, surfactant, and preservatives	13.3 mL
	Emit® Methotrexate Calibrators 0, 0.2, 0.5, 1, 1.5, 2 methotrexate, human serum, and preservatives (see below for concentrations)	six 1 mL vials*

Reagents A and B and calibrators are shipped in dry form. The indicated volume is that required for reconstitution.

†***The antibody titer and enzyme conjugate activity may vary from lot to lot.***

Note: Reagents A and B and calibrators are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

The Emit® Methotrexate Calibrators, when reconstituted, contain the following stated methotrexate concentrations:

Calibrators	0	0.2	0.5	1	1.5	2
Methotrexate (µmol/L)	0	0.2	0.5	1	1.5	2
Methotrexate (mol/L)	0	$2x10^{-7}$	$5x10^{-7}$	$1x10^{-6}$	$1.5x10^{-6}$	$2x10^{-6}$

For *in vitro* diagnostic use.

Precautions

- The human blood source material used to prepare this product has been tested and found nonreactive for human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2), nonreactive for hepatitis B surface antigen (HBsAg), and nonreactive for antibodies to hepatitis C virus (anti-HCV) when tested by licensed reagents. Because no known test method can offer complete assurance that products derived from human blood are pathogen-free, handle all materials of human origin as though they were potentially infectious. If exposed to solutions containing materials of human origin, the user should follow recommendations of the U.S. Occupational Safety and Health Administration.[3,4]
- Contains sodium azide as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

Preparation and Storage of Assay Components

To reconstitute Reagent A or B or the calibrators, remove the metal seal and mark the rubber stopper to identify it with the original vial. Remove the stopper and add the amount of distilled or deionized water listed in Table 1. Replace the stopper and gently swirl the vial to dissolve the contents. **After reconstitution, allow the reagents and calibrators to equilibrate at a room temperature of 20–25°C for the length of time stated in Table 1.** Alternatively, reconstitute the reagents and calibrators the day before they are to be used and refrigerate them overnight at 2–8°C. After the equilibration period, always store the reagents and calibrators refrigerated at 2–8°C when not in use and allow them to reach room temperature before use. Store them upright after reconstitution. Do not freeze or expose them to temperatures above 32°C.

To prepare the buffer solution, transfer the entire contents of the buffer concentrate vial to a clean, graduated container (plastic or glass). Rinse the concentrate bottle several times with distilled or deionized water to ensure complete transfer of material. Dilute accurately to 200 mL with distilled or deionized water. Invert several times to mix thoroughly.

Table 1 — Preparation, Storage, and Stability of Assay Components

Component	Storage Temp.	Recon. Volume	Minimum Recon. Time 20–25°C	Stability* Unopened	Stability* Prepared
Reagents A & B	2–8°C	3 mL	1 h	Exp. date	12 wk
Calibrators	2–8°C	1 mL	1 h	Exp. date	12 wk
Buffer	Unopened: 2–8°C Diluted: 20–25°C	Dilute to 200 mL	None	Exp. date	12 wk

Stability depends on handling assay components as directed.

5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. Acceptable anticoagulants are heparin, EDTA, and oxalate.
- Sample volume is instrument-dependent. Refer to the appropriate instrument application sheet for specific volumes.
- Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. These factors include dosage form, mode of administration, concomitant drug therapy, and biological variations affecting drug disposition.
- Store the serum or plasma refrigerated at 2–8°C and protected from light (methotrexate in solution is light sensitive). For transporting, maintain the sample temperature at 2–8°C.

6 Procedure

Materials Provided

Emit® Methotrexate Assay Kit, when prepared, contains:
Antibody/Substrate Reagent A
Enzyme Reagent B
Emit® Drug Assay Buffer Concentrate
Emit® Methotrexate Calibrators

Materials Required But Not Provided

Controls
Class A volumetric pipettes
Distilled or deionized water

Instruments

Siemens Healthcare Diagnostics provides instructions for using this assay on a number of chemistry analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for application sheets.

Analyzers must be capable of maintaining a constant reaction temperature, pipetting specimens/reagents and measuring enzyme rates precisely, timing the reaction accurately, and mixing reagents thoroughly.

The procedure for performing the Emit® Methotrexate Assay manually on the Syva® Lab System is described below.

Procedure

The Emit® Methotrexate Assay can be used to analyze samples containing 0.3–2600 µmol/L methotrexate; quantitation of concentrations less than 0.3 µmol/L is not recommended. Patient samples and calibrators containing 0.3–2 µmol/L methotrexate are assayed according to the primary protocol. Samples containing more than 2 µmol/L methotrexate can be brought within the range of the standard curve by using a calibrated pipette to serially dilute them 1:6 with buffer solution (see supplementary protocol).

Table 2 shows the tube configurations and dilution factors needed to bring high concentration samples into the assay range.

Table 2 — Tube Configuration and Dilution Factors

	Initial Sample Range (µmol/L) 0.3–2	1.8–12	10.8–72	65–430	390–2600
Protocol (brings sample into quantitation range)	Primary Protocol	One Additional Dilution	Two Additional Dilutions	Three Additional Dilutions	Four Additional Dilutions
Sample	Primary Tube (Tube A)	Tube A	Tube B	Tube C	Tube D
Sample Vol	NA	50 µL	50 µL	50 µL	50 µL
Buffer	None	250 µL	250 µL	250 µL	250 µL
Final Tube Label	A	B	C	D	E
Adjustment Factor*	Not Needed	6	36	216	1296

Note: This table shows the arrangement of tubes in the assay work rack corresponding to each dilution step. Tubes can be arranged to help monitor the number of dilutions performed. For instance, if a sample requires three additional dilutions to bring it within the quantitation range, the resulting configuration of tubes can be a reminder to use an adjustment factor of 216 to calculate results. Tubes are labeled alphabetically to assist in the detailed protocol explanation.

Use the adjustment factor to compute the actual methotrexate concentration of a sample when using additional dilutions. If three additional dilutions are needed to bring a sample into the quantitation range, the concentration quantitated from the standard curve is multiplied by 6^3 or 216 to account for the three additional 1:6 dilutions.

Setup

1. **Prepare Samples and Reagents.** Prepare all reagents according to the directions in Section 4. **Allow all reagents, samples, and materials to reach a room temperature of 20–25°C. Swirl the contents of all containers to mix reagents thoroughly just before use.**
2. **Prepare instruments.** Set up the instrument according to the operator's manual.

To prevent cross-contamination of samples and controls, assay each sample individually and complete all necessary dilution and assay steps before proceeding to the next sample.

1. **Set Up Tubes.** Set up four 2 mL disposable tubes in the work rack corresponding to positions B, C, D, and E as shown in Table 2. Using a calibrated pipette, add 250 µL buffer to each tube.
2. **Dilute Sample.** Using the calibrated pipette, aspirate 50 µL of calibrator, control, or sample and deliver this into Tube B (which contains 250 µL buffer).
3. Cover tube with cap or parafilm and mix gently by inversion.
4. **Dilute Sample Again.** Using the calibrated pipette, aspirate 50 µL of the diluted sample (Tube B) and deliver this into Tube C (which contains 250 µL buffer).
5. Cover tube with cap or parafilm and mix gently by inversion.
6. **Dilute Sample Again.** Using the calibrated pipette, aspirate 50 µL of the diluted sample (Tube C) and deliver this into Tube D (which contains 250 µL buffer).
7. Cover tube with cap or parafilm and mix gently by inversion.
8. **Dilute Sample Again.** Using the calibrated pipette, aspirate 50 µL of the diluted sample (Tube D) and deliver this into Tube E (which contains 250 µL buffer).
9. Cover tube with cap or parafilm and mix gently by inversion.
10. Transfer samples from Tubes A through E to appropriate instrument sample cup. Assay all specimens according to instrument protocol.
11. When a value for the specimen on the analyzer is between 0.3 and 2 µmol/L, multiply the observed value by the corresponding adjustment factor for the tube listed in Table 2.

Calibration

Prepare a new standard curve whenever a new set of reagents is used and recalibrate as indicated by control results (see Quality Control, below). The calibration sequence is 0, 1, 2, 3, 4, 5. If a new bottle of buffer is used, validate the system by running controls.

Quality Control

- Validate the standard curve by assaying multi-level controls. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the standard curve is validated, run patient samples.
- Follow government regulations or accreditation requirements for quality control frequency. At least once each day of use, analyze two levels of a Quality Control (QC) material with known methotrexate concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
- Refer to the instrument operator's manual(s) for appropriate instrument checks.

Daily Maintenance

Refer to the instrument operator's manual(s) for maintenance instructions.

7 Results

- Results are calculated automatically by the analyzers. No additional manipulation of data is required.
- This assay uses Math Model No. 2.
- Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.
- Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.
- The concentration of methotrexate in serum or plasma depends on the time of the last drug dose; mode of administration; concomitant drug therapy; sample condition; time of sample collection; and individual variations in absorption, distribution, biotransformation, and excretion. These parameters must be considered when interpreting results.[1]

8 Limitations

- Quantitation of samples with methotrexate concentrations less than 0.3 µmol/L (3×10^{-7} mol/L) is not recommended (see Section 6, Procedure).
- Aminopterin, an antineoplastic agent not usually administered concurrently with methotrexate, and 4-amino-4-deoxy-N^{10}-methylpteroic acid (APA), a minor metabolite of methotrexate, cross-react significantly with this assay.

9 Expected Values

The Emit® Methotrexate Assay accurately quantitates methotrexate concentrations in human serum or plasma containing 0.3–2600 µmol/L (3×10^{-7} – 2.6×10^{-3} mol/L) of methotrexate. A serum methotrexate concentration above 5 µmol/L (5×10^{-6} mol/L) at 24 hours after high-dose therapy generally indicates a risk of toxicity.[1] This maximum level is provided only as a guide; individual patient results should be interpreted in light of all clinical signs and symptoms.

10 Specific Performance Characteristics

Performance characteristics of the Emit® Methotrexate Assay are affected by all parameters of the measurement. The following information represents total system performance and should not be interpreted to pertain only to reagents.

Specificity

The Emit® Methotrexate Assay measures the total (protein-bound plus unbound) methotrexate concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested.

Aminopterin, an antineoplastic agent not usually administered concurrently with methotrexate, and 4-amino-4-deoxy-N^{10}-methylpteroic acid (APA), a minor metabolite of methotrexate, cross-react significantly with this assay.

The compounds listed in Table 3 do not interfere with the Emit® Methotrexate Assay at maximum pharmacological or physiological concentrations when tested in the presence of 1 µmol/L methotrexate.

Table 3 — Compounds That Do Not Interfere

Structurally Unrelated Compounds
Cyclophosphamide
Doxorubicin (Adriamycin)
5-Fluorouracil
Vinblastine
Vincristine
Structurally Related Compounds
Dihydrofolic Acid
Folic Acid
7-Hydroxymethotrexate
Leucovorin (citrovorum factor)
Methopterin
Trimethoprim

For additional information, contact the Technical Assistance Center.

Endogenous Substances

No clinically significant interference has been found in samples to which 800 mg/dL hemoglobin, 1000 mg/dL triglycerides, or 30 mg/dL bilirubin were added to simulate hemolytic, lipemic, or icteric samples.

Precision

The precision and accuracy values shown below were obtained from a typical test procedure. They are not intended to represent performance in all laboratories.

In clinical investigations, within-run precision was determined by 20 replicate analyses of the 1 µmol/L calibrator; between-run precision was determined by assaying a methotrexate control. Results follow.

Table 4 — Precision

Laboratory	Number of Replicates	Mean (µmol/L)	Coefficient of Variation (%)
Within-Run			
1	20	1	5.3
2	20	1	3.2
3	20	1	6.8
Between-Run			
2	49	1.63	5.6

Accuracy

Samples from patients receiving methotrexate were analyzed by the Emit® Methotrexate Assay and by at least one of two reference methods, either radioimmunoassay (RIA), or an enzymatic technique based on the inhibition of dihydrofolate reductase. The results were compared.

Table 5 — Comparative Analysis

	Lab 1 (RIA)	Lab 2 (RIA)	Lab 3 (Enzymatic)
Slope	0.96	0.98	0.97
Intercept (µmol/L)	-0.04	-0.07	-0.027
Mean (µmol/L)			
Emit®	3	5.6	4.5
Comparison Method	3.3	6.8	4.5
Correlation Coefficient	0.991	0.996	0.997
Number of Samples	104	98	100

11 Risk and Safety

H302 + H312, H319, H315, H334, H317, H412
P280, P273, P342 + P311, P302 + P312, P501

Danger!

Harmful if swallowed or in contact with skin. Causes serious eye irritation. Causes skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause an allergic skin reaction. Harmful to aquatic life with long lasting effects.

Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF ON SKIN: Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Sodium azide; 2-methyl-3(2h)-isothiazolone, hydrochloride

Safety data sheets (MSDS/SDS) available on siemens.com/healthcare

12 Bibliography

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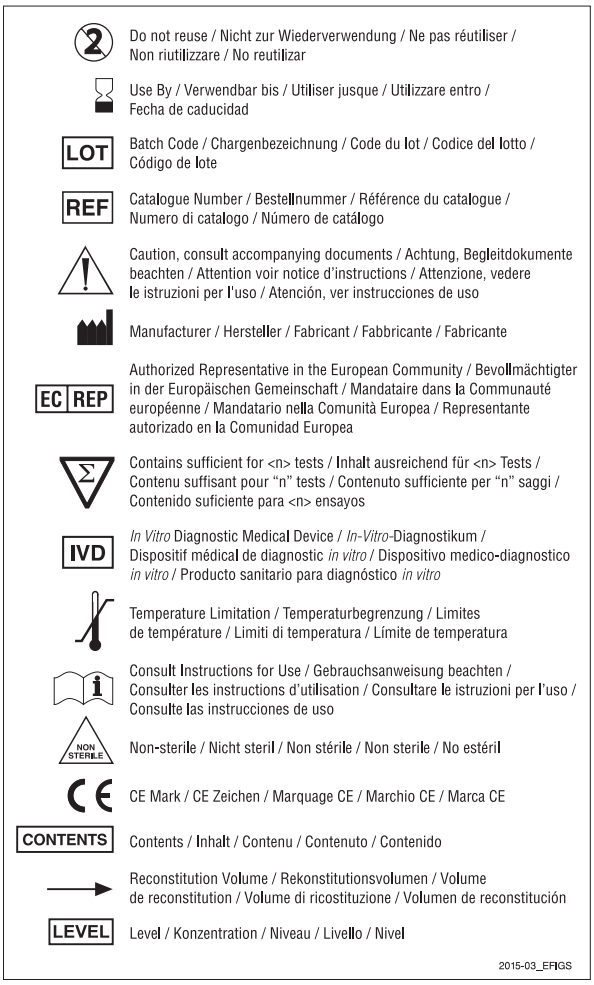
3. Biosafety in Microbiological and Biomedical Laboratories, JY Richmond, RW McKinney (eds). Atlanta, GA, U.S. Dept of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention; Bethesda, MD, National Institutes of Health, 1993.
4. Occupational Exposure to Bloodborne Pathogens; Final Rule. Federal Register. Part II; Department of Labor, Occupational Safety and Health Administration (OSHA); 29 CFR Part 1910.1030; Friday, December 6, 1991.

13 Symbols Key



For technical assistance, call Siemens Healthcare Diagnostics:

1-800-227-8994 in the USA

1-800-264-0083 in Canada

Outside the USA and Canada, call your local Siemens representative.

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