SIEMENS

Syva®

Emit® 2000 Theophylline Assay

See shaded sections:
Updated information from 2015-04 version.

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Theophylline Assay

1 Intended Use

The Emit® 2000 Theophylline Assay is a homogeneous enzyme immunoassay intended for use in the quantitative analysis of theophylline in human serum or plasma. Emit® 2000 assays are designed for use with most chemistry analyzers (see Section 6, Procedure, Instruments).

2 Summary and Explanation of the Test

The physiological effects of the antiasthmatic drug theophylline correlate better with the drug's concentration in serum than with dosage. Since serious toxic effects of theophylline are related to the serum concentration and are not always preceded by minor adverse symptoms, serum theophylline monitoring helps to avoid serious toxicity.[1–5]

When theophylline is used to treat acute symptoms, monitoring serum concentrations allows the physician to adjust the dosage regimen to compensate for interpatient variations in the theophylline elimination rate.[1] The chronic treatment of asthma and other bronchospastic diseases also requires individualization of the theophylline dosage to maintain serum concentrations within the therapeutic range.[2,3] A theophylline dosage generally can be maintained without further monitoring for six months in rapidly growing children and for twelve months in other patients. Changes in concurrent drug therapy, variations in drug elimination, or the appearance of side effects, uncontrolled symptoms, or altered drug clearance signal the need for measuring the serum theophylline concentration.[1,3]

Methods historically used to monitor serum theophylline concentrations include gas-liquid chromatography, high-performance liquid chromatography, and immunoassay.[1,5,6]

3 Principle

The Emit® 2000 assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids.[7] 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
4P019UL	**Emit® 2000 Theophylline Assay**	
	Antibody/Substrate Reagent 1 mouse monoclonal antibodies reactive to theophylline (57 µg/mL),* glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), preservatives, including 0.1% sodium azide, and stabilizers	28 mL
	Enzyme Reagent 2 theophylline labeled with bacterial glucose-6-phosphate dehydrogenase (0.24 U/mL),* Tris buffer, preservatives, including 0.1% sodium azide, and stabilizers	14 mL

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

Note: Reagents 1 and 2 are provided as a matched set. They should not be interchanged with components of kits with different lot numbers.

For *in vitro* diagnostic use.

Precautions

- 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.
- This kit contains streptomycin sulfate. Please dispose of appropriately.

Preparation and Storage of Assay Components
Emit® 2000 Theophylline Assay

The Emit® 2000 Theophylline Assay reagents are provided ready to use and may be used directly from the refrigerator. Close the reagent vials when not in use. Always return the reagent screw caps to their original vials.

Store reagents at 2–8°C (36–46°F), upright, and with screw caps tightly closed when not in use. Reagents are stable until the expiration date printed on the label if stored as directed. Do not freeze reagents or expose them to temperatures above 32°C. **Improper storage of reagents can affect assay performance.**

5 Specimen Collection and Preparation

- Each assay requires serum or plasma. Whole blood cannot be used. The anticoagulants heparin, citrate, oxalate, and EDTA have been tested and may be used with this assay. Some sample dilution may occur when samples are collected in tubes containing citrate anticoagulant. The amount of dilution and the possible need to correct for it should be considered when interpreting assay results for these samples.
- Sample volume is instrument-dependent. Refer to the appropriate instrument protocol sheet for specific volumes.
- Store the serum or plasma refrigerated at 2–8°C. For transporting, maintain the sample temperature at 2–8°C. Samples can be stored refrigerated at 2–8°C for up to one month or stored frozen for up to three months.
- 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.[1–5] Patient intake of caffeinated beverages does not need to be restricted.
- Human serum or plasma samples should be handled and disposed of as if they were potentially infectious.

6 Procedure

Materials Provided
Emit® 2000 Theophylline Assay
Reagent 1
Reagent 2

Materials Required But Not Provided
4P109UL Emit® 2000 Theophylline Calibrators (0, 2.5, 5, 10, 20, 40 µg/mL)

Multi-level commercial controls

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.

Calibration

Recalibrate whenever a new lot of reagents is used or as indicated by control results (see Quality Control, below). If a new set of reagents with the same lot number is used, validate the system by assaying controls.

Quality Control

1. Validate the calibration by assaying multi-level controls. Commercial controls are available for this purpose. Ensure that control results fall within acceptable limits as defined by your own laboratory. Once the calibration is validated, run samples.
2. 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 Theophylline concentrations. Follow your laboratory internal QC procedures if the results obtained are outside acceptable limits.
3. Refer to the instrument operator's manual for appropriate instrument checks.

Diluting High Concentration Samples

To estimate theophylline concentrations above the assay range, patient samples containing more than 40 µg/mL (222 µmol/L) theophylline may be diluted with one or two parts distilled or deionized water or Emit® 2000 Theophylline Calibrator 0. After diluting the sample, repeat the entire assay sequence and multiply the results by the dilution factor. Some analyzers dilute and retest high concentration samples automatically. See the appropriate protocol sheet for instructions.

Daily Maintenance

Refer to the instrument operator's manual 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. 1.
- Consult the appropriate instrument operator's manual and protocol sheet for complete instructions.
- 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 factors that can influence the relationship between theophylline serum or plasma concentrations and clinical response include the type and severity of bronchial constriction, age, smoking, diet, general state of health, and use of other drugs.[2,3]
- The concentration of theophylline in serum or plasma depends on the time of the last drug dose; dosage form; 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.[2–5]

8 Limitations

This assay has no specific limitations.

9 Expected Values

The Emit® 2000 Theophylline Assay accurately quantitates theophylline concentrations in human serum or plasma containing 2.5–40 µg/mL (14–222 µmol/L) theophylline. In most patients, theophylline serum concentrations of 10–20 µg/mL (56–111 µmol/L) effectively suppress chronic asthmatic and other bronchospastic symptoms.[2–5] Serum concentrations of 5–10 µg/mL (28–56 µmol/L) theophylline reportedly control apneic spells in neonates without causing apparent side effects.[2–4] Further, peak concentrations above 20 µg/mL (111 µmol/L) are often associated with toxicity.[2–5]

Note: To convert from µg/mL to µmol/L theophylline, multiply by 5.55.

For effective treatment, some patients may require serum levels outside this range. Therefore, the expected range is provided only as a guide, and individual patient results should be interpreted in light of other clinical signs and symptoms (see Section 7, Results).

10 Specific Performance Characteristics

The data appearing in this section were collected on the Roche Diagnostics (RD)/Hitachi 704 Analyzer and the COBAS MIRA® Chemistry System. Performance characteristics (including calibration stability) are available for a variety of analyzers. Contact the Technical Assistance Center in the USA or your local Siemens representative for information for specific analyzers.

The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents. Performance may vary depending on the instrumentation used.

Specificity

The Emit® 2000 Theophylline Assay measures the total (protein-bound plus unbound) theophylline concentration in serum or plasma. Compounds whose chemical structure or concurrent therapeutic use would suggest possible cross-reactivity have been tested.

The compounds listed in Table 1 do not interfere with the Emit® 2000 Theophylline Assay when tested in the presence of 10 µg/mL theophylline. Levels tested were at or above maximum physiological or pharmacological concentrations.

Table 1 — Compounds That Do Not Interfere

Compound	Concentration Tested (µg/mL)	Compound	Concentration Tested (µg/mL)
Caffeine	35	3-Methylxanthine	100
8-Chlorotheophylline	25	7-Methylxanthine	100
1,3-Dimethyluric Acid	100	Paraxanthine	50
Dyphylline	100	Phenobarbital	100
Ephedrine	5	Theobromine	100
Hypoxanthine	100	1,3,7-Trimethyluric Acid	100
1-Methyluric Acid	100	Urea	1000
3-Methyluric Acid	200	Uric Acid	200
1-Methylxanthine	30	Xanthine	100

Note: The compound 3-isobutyl-1-methylxanthine interferes in this assay. The compound is not a naturally occurring xanthine or known metabolite, but some laboratories use it as an internal standard in chromatographic procedures.[8]

For additional information, contact Siemens.

Sensitivity

The sensitivity level of Emit® 2000 Theophylline Assay is 0.75 µg/mL. This level represents the lowest measurable concentration of theophylline that can be distinguished from 0 µg/mL with a confidence level of 95%.

Endogenous Substances

No clinically significant interference has been found in samples to which 800 mg/dL hemoglobin, 750 mg/dL triglycerides, or 30 mg/dL bilirubin were added to simulate hemolytic, lipemic, or icteric samples.

Calibration Stability

In-house and field studies have shown calibration stability of more than two weeks. When proper reagent handling, instrument maintenance, and operating procedures are followed, the calibration should remain stable for at least two weeks.

Precision

The precision values shown in Tables 2 and 3 were obtained in clinical trials using the RD/Hitachi 704 and COBAS MIRA® analyzers.

Within-run precision was determined using tri-level commercial controls.

Table 2 — Within-Run Precision for Emit® 2000 Theophylline Assay on the RD/Hitachi 704 and COBAS MIRA® Analyzers

Site/ Analyzer	Number of Replicates	Mean (µg/mL)	Coefficient of Variation (%)
Site 1	20	5.2	1.2
RD/Hitachi 704	20	16	2.4
	20	31	3.3
Site 2	20	5.2	4.6
COBAS MIRA®	20	16.1	7
	20	30.5	7.2
Site 3	20	5.4	4.8
COBAS MIRA®	20	17	6.8
	20	33	7.9

Between-run precision was determined over several days using tri-level commercial controls.

Table 3 — Between-Run Precision for Emit® 2000 Theophylline Assay on the RD/Hitachi 704 and COBAS MIRA® Analyzers

Site/ Analyzer	Length of Study (days)	Number of Replicates	Mean (µg/mL)	Coefficient of Variation (%)
Site 1	60	60	5.2	2.9
RD/Hitachi 704		60	16.4	4.2
		60	32.5	6.6
Site 2	42	60	5.4	5.2
COBAS MIRA®		60	16.1	6.9
		60	32.3	8.4
Site 3	11	60	5.3	4.2
COBAS MIRA®		60	16.5	6.3
		60	33.5	7.8

Accuracy

Samples from patients receiving theophylline were analyzed by the Emit® 2000 Theophylline Assay on the RD/Hitachi 704 or COBAS MIRA® analyzers, and the results were compared to two alternative methods: Emit® Theophylline Assay on the RD/Hitachi 704 analyzer and fluorescence polarization immunoassay (FPIA). Data are summarized in Table 4.

Table 4 — Comparative Analysis

	Emit® 2000* vs FPIA	Emit® 2000* vs Emit®*	Emit® 2000† vs FPIA
Slope	1.04	0.92	1.10
Intercept (µg/mL)	-0.06	0.21	-0.52
Mean (µg/mL)			
Emit® 2000 Assay	16.4	16.4	14.1
Comparison Method	15.8	17.6	13.3
Standard Error of the Estimate (µg/mL)	1.09	0.97	0.97
Correlation Coefficient	0.99	0.99	0.99
Number of Samples	104	104	128

****This assay was performed on the RD/Hitachi 704 analyzer.***

†This assay was performed on the COBAS MIRA® analyzer.

11 Risk and Safety

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

12 Bibliography

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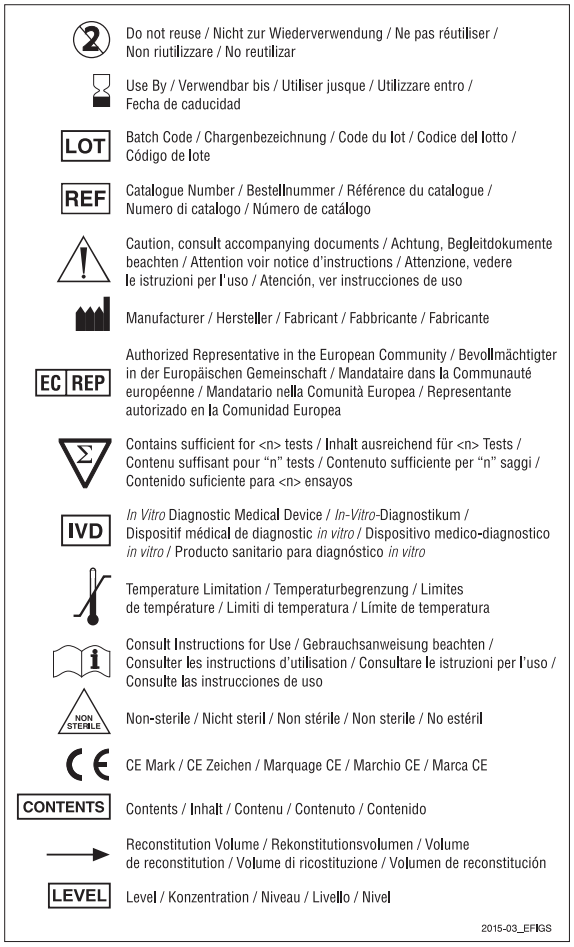
13 Symbols Key



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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