Theophylline Assay

Use in the quantitative analysis of theophylline in human serum or plasma. These reagents are used for the analysis of specific compounds in biological fluids. The assay is based on the appearance of side effects, uncontrolled symptoms, or altered drug clearance signal the need for chromatography, high-performance liquid chromatography, and immunoassay.

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. These reagents are packaged specifically for use on a variety of AU® Clinical Chemistry Systems.

2 SUMMARY

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.

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. The chronic treatment of asthma and other bronchospastic diseases also requires individualization of the theophylline dosage to maintain serum concentrations within the therapeudic range.

Generally, a theophylline dosage 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.

Methods historically used to monitor serum theophylline concentrations include gas-liquid chromatography, high-performance liquid chromatography, and immunoassay.

3 METHODOLOGY

The Emit® 2000 Theophylline Assay is a homogeneous enzyme immunoassay technique used for the analysis of specific compounds in biological fluids. 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

Reagents contain the following substances:

Mouse monoclonal antibodies reactive to theophylline (57 µg/mL), glucose-6-phosphate (22 mM), nicotinamide adenine dinucleotide (18 mM), theophylline labeled with glucose-6-phosphate dehydrogenase (0.24 U/mL), 0.1% sodium azide, Tris buffer, preservatives, and stabilizers.

Precautions

• For in vitro diagnostic use.
• Contains nonsterile mouse monoclonal antibodies.
• Assay components contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
• This kit contains streptomycin sulfate. Please dispose of appropriately.
• Do not use the kit after the expiration date.
• Turbid or yellow reagents may indicate contamination or degradation and must be discarded.

Preparation of Reagents

The Emit® 2000 Theophylline Assay reagents are provided ready to use; no preparation is necessary.

Storage of Assay Components

• Improper storage of reagents can affect assay performance.
• When not in use, store reagents upright at 2–8°C and with screw caps tightly closed.
• Unopened reagents are stable until the expiration date printed on the label if stored upright at 2–8°C.
• Do not freeze reagents or expose them to temperatures above 32°C.

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 Application 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.
• 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

Emit® 2000 Theophylline Calibrators

Multi-level commercial controls

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

• 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.
• Refer to the instrument User’s Guide for appropriate instrument checks and maintenance instructions.
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 analyzer User’s Guide or appropriate Application Sheet for instructions.

Evaluation and Interpretation of Results
• This assay uses Math Model No. 1.
• Results are automatically calculated; no additional manipulation of data is required.
• 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

7 LIMITATIONS OF THE PROCEDURE
The compound 3-isobutyl-1-methylxanthine interferes with 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.9

8 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.2,4 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 6, Procedure, Evaluation and Interpretation of Results).

9 SPECIFIC PERFORMANCE CHARACTERISTICS
The information presented in this section is based on Emit® 2000 Theophylline Assay studies performed on the AU4000®/AU600® Clinical Chemistry System. Refer to the Application Sheets for other AU Clinical Chemistry Systems and for additional information. Results may vary due to analyzer-to-analyzer differences. The following performance characteristics represent total system performance and should not be interpreted to pertain only to reagents.

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.

Precision
Within-run precision was determined by assaying 20 replicates of each level of a tri-level control. Table 1 summarizes the data.

Table 1 — Within-Run Precision
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration Tested (µg/mL)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>100</td>
<td>2.7</td>
</tr>
<tr>
<td>8-Chlorotheophylline</td>
<td>25</td>
<td>3.0</td>
</tr>
<tr>
<td>1,3-Dimethyluric acid</td>
<td>100</td>
<td>6.6</td>
</tr>
<tr>
<td>Dyphylline</td>
<td>100</td>
<td>2.7</td>
</tr>
<tr>
<td>Ephedrine</td>
<td>5</td>
<td>6.6</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>100</td>
<td>2.7</td>
</tr>
<tr>
<td>1-Methyluric acid</td>
<td>100</td>
<td>6.6</td>
</tr>
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<td>1-Methylxanthine</td>
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Total precision was calculated according to NCCLS guideline EPS-T2 using data collected from controls run in duplicate twice daily over twenty (20) days. Table 2 summarizes the data.

Table 2 — Total Precision
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Comparative Analysis
Samples from patients were analyzed on the TDx analyzer and on the AU600 Clinical Chemistry System. A summary of the results is as follows:

Table 3 — Compounds That Do Not Interfere
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Note: The compound 3-isobutyl-1-methylxanthine interferes with 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.9

Sensitivity
The sensitivity level of the 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%.

Calibration Stability
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.

10 REFERENCES
For technical assistance:

Beckman Coulter customers contact their technical assistance center.
1-800-223-0130

Siemens Healthcare Diagnostics customers contact their technical assistance center.
1-800-227-8994 in the USA
1-800-264-0083 in Canada

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