THEO

Theophylline

cobas®

Therapeutic drug monitoring

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20737917 322	Theophylline (225 tests)	System-ID 07 3791 7	COBAS INTEGRA 400 plus COBAS INTEGRA 800
03375790 190	Preciset TDM I Calibrators A-F (6 × 1 × 5 mL) Diluent (1 × 10 mL)	System-ID 07 6830 8	
04521536 190	TDM Control Set Level I (2 × 5 mL) Level II (2 × 5 mL) Level III (2 × 5 mL)	 System-ID 07 6900 2 System-ID 07 6901 0 System-ID 07 6902 9	
20720720 322	COBAS FP Sample Dilution Reagent II (1 × 200 mL)	System-ID 07 2072 0	

English

System information

Test THEOM, test ID 0-391

Intended use

In vitro diagnostic test for the quantitative determination of theophylline in serum or heparinized plasma on COBAS INTEGRA systems.

Summary

Theophylline (1,3-dimethylxanthine), a bronchodilator, is widely used to treat patients with asthma, apnea (temporary asphyxia), and other obstructive lung diseases. The monitoring of serum theophylline levels is important because there are wide discrepancies between drug dosage and serum concentrations among patients receiving identical doses.[1]

Test principle

Fluorescence polarization

COBAS INTEGRA therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using the principle of fluorescence polarization. When a fluorescent molecule, or fluorophore, is irradiated with light of the proper wavelength (the excitation wavelength) some of the light is absorbed. Within a few nanoseconds the absorbed light is emitted, although at a longer wavelength (the emission wavelength). Whether or not the emitted light is polarized depends on the freedom of the fluorophore to rotate in solution. A small molecule, such as fluorescein, can rotate rapidly before light emission occurs, resulting in depolarization of the emitted light. In contrast, a fluorescent macromolecule, such as a fluorescein-labeled protein, will rotate much more slowly. Thus, in the time frame between excitation and emission, the macromolecule will have rotated only very slightly and the emitted light will be polarized.[2] Fluorescence polarization is a reproducible function of the drug concentration, and is suitable for the quantitative determination of drug concentrations in serum for the purpose of therapeutic drug monitoring.

Surface active agents are used to ensure dissociation of the drug from serum proteins and to prevent nonspecific binding of the tracer.

Reagents - working solutions

R1 Antibody reagent
Anti-theophylline monoclonal antibody (mouse) in buffer, pH 7.5, with stabilizer and preservative.

SR Tracer reagent
Fluorescein-labeled theophylline derivative in buffer, pH 7.5, with stabilizer, surfactant, and preservative.

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C	12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C	26 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable:
Unhemolyzed serum
Unhemolyzed heparinized plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Usual sampling time varies dependent upon route of delivery and desired measurement of peak or trough values.[3] Specimens should be tested within 8 hours of collection if kept at room temperature. If specimens must be stored for later testing, they may be kept at 2-8 °C for up to 1 week or at -20 °C for up to 60 days.[4] Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

COBAS FP Sample Dilution Reagent (SDR II), Cat. No. 20720720 322
The SDR II is placed as special diluent in its predefined rack position and is stable for 7 days on-board COBAS INTEGRA 400 plus/800 analyzers.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm
Reading cycle blank/test		29/45
Unit		µg/mL

Pipetting parameters

	Diluent (H_2O)

R1	130 µL	10 µL
Sample	3 µL	5 µL
Special diluent SDR II	27 µL	
SR	18 µL	10 µL
Total volume	203 µL	

COBAS INTEGRA 800 test definition

Measuring mode		FP
Reaction mode		R1-SDR/S-SR
Wavelength	excitation	485 nm
	emission	515 nm
Reading cycle blank/test		40/60
Unit		µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	130 µL	10 µL
Sample	3 µL	5 µL
Special diluent SDR II	27 µL	
SR	18 µL	10 µL
Total volume	203 µL	

Calibration

Calibrators	Preciset TDM I Calibrators A-F
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Deviation low/high	< 10 % at ≥ 2.5 µg/mL (≥ 13.9 µmol/L)
Calibration interval	Each lot, every 20 weeks, and as required following quality control procedures

A calibration curve must be prepared using the Preciset TDM I calibrators. Calibrators must be placed from the highest concentration (F) first, to the lowest (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The Preciset TDM I calibrators are prepared to contain known quantities of theophylline in normal human serum and are traceable to USP reference standards.

Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

Quality control

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Note

Controls should be assayed within 2 hours after placing on-board the instrument.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/mL × 5.55 = µmol/L

Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Specimens with assay values greater than the highest calibrator will be flagged by the system and must be repeated after appropriate dilution of the original sample with the Preciset TDM I Diluent (0 µg/mL). Specimens with high fluorescent backgrounds or those giving polarization values greater than the zero calibrator will also be flagged by the system.

Serum/plasma

Criterion: Recovery within ± 10 % of initial value at a theophylline concentration of 15.5 µg/mL (86 µmol/L).

Icterus:[5] No significant interference up to a bilirubin concentration of 487 µmol/L or 28.5 mg/dL.

Hemolysis:[5] No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Lipemia:[5] No significant interference up to a triglycerides concentration of 1730 mg/dL.

Total protein: No significant interference up to a total protein concentration of 12 g/dL.

For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.
Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

COBAS INTEGRA 400 plus analyzer:
0.18-40 µg/mL (1.0-222 µmol/L)

COBAS INTEGRA 800 analyzer:
0.16-40 µg/mL (0.89-222 µmol/L)

Lower limits of measurement

Lower detection limit of the test:

COBAS INTEGRA 400 plus analyzer:
0.18 µg/mL (1.00 µmol/L)

COBAS INTEGRA 800 analyzer:
0.16 µg/mL (0.89 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

Expected values

Various methodologies have been used to evaluate theophylline preparations and routes of administration,[6] to study pharmacokinetics of the drug,[7] and to define the relationship between serum concentration and the drug’s therapeutic and toxic effects.[8] For most patients, the range of 10-20 µg/mL (55.5-111 µmol/L) suppresses chronic asthmatic symptoms.[1,9,10,11] Wide discrepancies between drug dosage and serum concentrations were observed among patients receiving identical doses.[1] A major factor accounting for the variability is individual variation in the rate of theophylline metabolism and elimination.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using controls in accordance with the NCCLS EP5-T2[12] requirements with repeatability (n = 80) and intermediate precision (2 aliquots per run, 2 runs per day, 20 days).The following results were obtained on a COBAS INTEGRA 400 analyzer.

Repeatability	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Level 1	5.4 (30.0)	0.09 (0.50)	1.6
Level 2	14.2 (78.8)	0.28 (1.55)	1.9
Level 3	22.5 (125)	0.41 (2.28)	1.8

Intermediate precision	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Level 1	5.4 (30.0)	0.14 (0.78)	2.6
Level 2	14.2 (78.8)	0.38 (2.11)	2.6
Level 3	22.5 (125)	0.64 (3.55)	2.8

Method comparison

Theophylline values for human serum samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Theophylline reagent (y) were compared with those determined using a commercially available FPIA method (x).

		FPIA
Number of samples		138
Range of values	min.	0.16 µg/mL
	max.	36.3 µg/mL
Slope		1.049
Intercept		-0.111 µg/mL
Correlation coefficient		0.997

Analytical specificity

The following cross-reactive substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with theophylline at 16.4 µg/mL (91.0 µmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.[13] The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

$$\text{Cross-reactivity (\%)} = \frac{100 \times (\text{analytical result - analyte concentration})}{\text{concentration of interferent}}$$

Drug	Level tested µg/mL	Cross-reactivity %
8-Chlorotheophylline	200	8.4
1,7-Dimethylxanthine	150	5.4
3-Methylxanthine	150	3.4
Theobromine	197	1.4
Caffeine	150	0.8
Doxofylline	160	0.4
Diphenhydramine	10	ND

ND = Not Detectable

In a similar study, the following structurally related or potentially co-administered compounds were tested on the COBAS FARA II analyzer using normal human serum spiked with theophylline at 15.3 µg/mL (84.9 µmol/L).

Drug	Level tested µg/mL	Cross-reactivity %
Aminophylline	15	75.6
1,3-Dimethyluric acid	672	< 0.6
7-β-Hydroxypropyl theophylline	200	< 0.5
Dihydroxypropyl theophylline	200	< 0.2
β-Hydroxyethyl theophylline	200	< 0.2
Acetaminophen	200	ND
Allopurinol	50	ND
Ephedrine	12	ND
Epinephrine	16	ND
Hypoxanthine	149	ND
Isoproterenol	50	ND
1-Methyluric acid	700	ND
Phenobarbital	400	ND
Phenylbutazone	450	ND
Phenytoin	200	ND
Uric acid	210	ND

ND = Not Detectable

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

1 Jackson FR, Garrido R, Silverman HI, et al. Blood levels following oral administration of theophylline preparations. Ann Allergy 1973;31:413-419.

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4 Committee on patient preparation and specimen handling. Clinical Laboratory Handbook for Patient Preparation and Specimen Handling. Fascicle IV. Skokie, IL: College of American Pathologists, 1985.

5 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

6 Truitt EG Jr, McKusick VA, Krantz C. Theophylline blood levels after oral, rectal, and intravenous administration and correlation with diuretic action. J Pharmcol Exp Ther 1950;100(3):309-315.

7 Mitenko PA, Ogilvie RI. Pharmacokinetics of intravenous theophylline. Clin Pharm Therapeutics 1973;14:509-513.

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9 Jenne JW, Wyze E, Rood FS, et al. Pharmacokinetics of theophylline: Application to adjustment of the clinical use of aminophylline Clin Pharmacol Ther 1972;13:349-360.

10 Weinberger MM, Bronsky EA. Evaluation of oral bronchodilator therapy in asthmatic children. J Pediatr 1974;84:421-427.

11 Weinberger MM, Riegelman S. Rational use of theophylline for bronchodilation. N Engl J Med 1974;291:151-153.

12 National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.

13 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

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Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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