THEO2

cobas®

ONLINE TDM Theophylline

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08253153190	08253153500	ONLINE TDM Theophylline 100 tests	System-ID 2109 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

03375790190	Preciset TDM I CAL A-F (1 x 5 mL) Diluent (1 x 10 mL)	Codes 20691-20696	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 20310 Code 20311 Code 20312	

English

System information

THEO2: ACN 21090

Intended use

In vitro test for the quantitative determination of theophylline in serum and plasma on **cobas c** systems.

Summary

Theophylline measurements, performed with this assay in human serum and plasma, are used in monitoring theophylline therapy to ensure appropriate therapy.

Theophylline (1,3-dimethylxanthine) is an effective bronchodilator and it can be used to treat asthma, chronic obstructive pulmonary disease and chronic bronchitis; it can also be used for the treatment of left ventricular and congestive cardiac failure.[1,2,3,4,5,6,7] It has a narrow therapeutic index and potentially severe concentration-dependent side effects. The dose of theophylline required to achieve therapeutic concentrations varies among patients, largely because of differences in clearance.[8] Increased clearance is seen in children (1–16 years) and in cigarette and marijuana smokers. Concurrent administration of drugs which increase P450 activity, such as phenytoin, phenobarbitone, or rifampicin, increases metabolic breakdown. Reduced clearance is found in liver disease, pneumonia, heart failure, viral infections and is also seen with several drugs which interfere with CYP1A2 function, such as erythromycin, quinolone antibiotics, allopurinol, cimetidine, serotonin uptake inhibitors, zileuton, and with some vaccinations (influenza immunizations).[8]

Because of these variations in clearance, individualization of theophylline dosage is required, and plasma concentrations should be monitored.[8] For sustained-release theophyllines, the serum concentration should be obtained in the middle of the dosing interval (3–5 days after initiation of theophylline) and then 2 days after initiation of any factor known to affect theophylline clearance significantly. If patients experience signs and symptoms of toxicity (e.g., severe headache, tachycardia, nausea and vomiting), theophylline should be discontinued and a serum concentration obtained. Monitoring of serum theophylline concentration is essential to ensure that toxic concentrations are avoided.[5]

Test principle

The assay is based on the kinetic interaction of microparticles in a solution (KIMS). Theophylline antibody is covalently coupled to microparticles and the drug derivative is linked to a macromolecule. The kinetic interaction of microparticles in solutions is induced by binding of drug-conjugate to the antibody on the microparticles and is inhibited by the presence of theophylline in the sample. A competitive reaction takes place between the drug conjugate and theophylline in the serum sample for binding to the theophylline antibody on the microparticles. The resulting kinetic interaction of microparticles is indirectly proportional to the amount of drug present in the sample.

Reagents - working solutions

R1 Theophylline conjugate; piperazine-N,N'-bis (ethanesulfonic acid) (PIPES) buffer, pH 7.2; preservative

R3 Anti-theophylline antibody (mouse monoclonal); latex microparticle; 3-(N-morpholino) propane sulfonic acid (MOPS) buffer, pH 7.5; stabilizer; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks

Do not freeze.

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.
Serum: Collect serum using standard sampling tubes
Plasma: K_2- or K_3-EDTA, sodium citrate, or sodium, lithium or ammonium heparin plasma.

Stability:[9]	1 week capped at 2-8 °C
	60 days capped at -20 °C (± 5 °C)

Freeze only once.

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.

See the limitations and interferences section for details about possible sample interferences.

Invert thawed specimens several times prior to testing.

Usual sampling time varies dependent upon desired measurement of peak or trough values.[10]

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

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General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

Test definition

Reporting time	10 min		
Wavelength (sub/main)	800/600 nm		
Reagent pipetting		Diluent (H_2O)	
R1	63 µL	–	
R3	60 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	1.3 µL	–	–
Increased	1.3 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators (full calibration)	S1-6: Preciset TDM I calibrators
Calibrators (2-point calibration)	S2: Preciset TDM I-B S5: Preciset TDM I-E
Calibration mode	Non-linear
Calibration frequency **cobas c** 303 analyzer	2-point calibration - every 7 days on-board Full calibration - after reagent lot change - every 6 weeks during shelf life - as required following quality control procedures
Calibration frequency **cobas c** 503 analyzer	Full calibration - after reagent lot change - every 6 weeks during shelf life - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against USP reference standards.[11] The calibrators are prepared to contain known quantities of theophylline in normal human serum.

Quality control

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks.

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.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample in the unit µg/mL (µmol/L).

Conversion factor:[12] µg/mL x 5.55 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at theophylline levels of approximately 5 and 15 µg/mL (27.8 and 83.3 µmol/L).

Icterus:[13] No significant interference up to an I index of 50 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 50 mg/dL or 855 µmol/L).

Hemolysis:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 1000 mg/dL or 621 µmol/L).

Lipemia (Intralipid):[13] No significant interference up to an L index of 300. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Triglycerides: No significant interference from triglycerides up to a concentration of 1000 mg/dL (11.3 mmol/L).

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 100 IU/mL.

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

Theobromine: No significant interference up to 49 µg/mL theobromine. Concentrations above this toxic level may result in negative bias of > 10 %.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[14]

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 c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.8-40.0 µg/mL (4.4-222 µmol/L)

Manually dilute samples above the measuring range 1 + 1 with the Preciset TDM I diluent (0 µg/mL) and reassay. Multiply the result by 2 to obtain the specimen value.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.8 µg/mL (4.4 µmol/L)
Limit of Detection	= 0.8 µg/mL (4.4 µmol/L)
Limit of Quantitation	= 2.0 µg/mL (11.1 µmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration theophylline samples.

Expected values

Various methodologies have been used to evaluate theophylline preparations and routes of administration,[15] to study pharmacokinetics of the drug,[16] and to define the relationship between serum concentration and

the drug's therapeutic and toxic effects.[17] For most patients, the range of 10 to 20 µg/mL (55.5 to 111 µmol/L) suppresses chronic asthmatic symptoms.[18,19,20,21] Wide discrepancies between drug dosage and serum concentrations were observed among patients.[15,18] A major factor accounting for the variability is individual variation in the rate of theophylline metabolism and elimination.

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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 analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* µg/mL	*SD* µg/mL	*CV* %
TDMC1[a)]	5.78	0.0257	0.4
TDMC2[b)]	14.8	0.0900	0.6
TDMC3[c)]	31.8	0.397	1.2
Human serum 1	2.14	0.0168	0.8
Human serum 2	9.90	0.0704	0.7
Human serum 3	15.1	0.102	0.7
Human serum 4	19.8	0.189	1.0
Human serum 5	33.5	0.604	1.8

Intermediate precision	*Mean* µg/mL	*SD* µg/mL	*CV* %
TDMC1[a)]	5.81	0.101	1.7
TDMC2[b)]	15.0	0.213	1.4
TDMC3[c)]	31.7	0.579	1.8
Human serum 1	2.21	0.0648	2.9
Human serum 2	9.90	0.135	1.4
Human serum 3	15.1	0.198	1.3
Human serum 4	19.8	0.282	1.4
Human serum 5	33.5	0.672	2.0

a) TDM Control Set Level I

b) TDM Control Set Level II

c) TDM Control Set Level III

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

Theophylline values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 74

Passing/Bablok[22]	Linear regression
y = 0.956x + 0.661 µg/mL	y = 0.952x + 0.711 µg/mL
τ = 0.990	r = 0.999

The sample concentrations were between 0.87 and 38.5 µg/mL.

Theophylline values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 73

Passing/Bablok[22]	Linear regression
y = 0.985x - 0.0434 µg/mL	y = 0.998x - 0.178 µg/mL
τ = 0.988	r = 0.999

The sample concentrations were between 1.56 and 38.6 µg/mL.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration tested (µg/mL)**	**% cross-reactivity**
Aminophylline	15	79.6
8-Chlorotheophylline	200	5.97
1,7-Dimethylxanthine	150	5.24
3-Methylxanthine	150	2.73
Ephedrine	12	1.00
Acetaminophen	200	< 1.0
Allopurinol	50	< 1.0
Caffeine	150	< 1.0
Dihydroxypropyl theophylline	200	< 1.0
Diphenhydramine	10	< 1.0
Epinephrine	16	< 1.0
β-Hydroxyethyl theophylline	200	< 1.0
7-β-Hydroxypropyl theophylline	200	< 1.0
Hypoxanthine	150	< 1.0
Isoproterenol	50	< 1.0
1-Methyluric acid	400	< 1.0
Phenobarbital	200	< 1.0
Phenylbutazone	400	< 1.0
Uric acid	210	< 1.0
1,3-Dimethyluric acid	700	< 0.1
Phenytoin	200	< 0.1

Tests were performed on 15 drugs. No significant interference with the assay was found.

Acetaminophen	Doxycycline (tetracycline)
Acetyl cysteine	Ibuprofen
Acetylsalicylic acid	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H_2O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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 for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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