ACET2

cobas®

ONLINE TDM Acetaminophen Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056684190	08056684500	ONLINE TDM Acetaminophen Gen.2 (500 tests)	System-ID 2004 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

07007515190	ACET2 calibrator (1 x 2 mL)	Code 20670	
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	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

For **cobas c** 303 analyzer:

ACET2: ACN 20040

For **cobas c** 503/703 analyzers:

ACET-2: ACN 20042

Intended use

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

Summary

Acetaminophen measurements performed with this assay, in human serum and plasma, are used as an aid in identifying acetaminophen intoxication, determining the need for treatment with N-Acetylcysteine to minimize toxic drug effects.

Acetaminophen is a widely used analgesic and antipyretic found in a number of over-the-counter and prescription products. The pharmacologic actions of acetaminophen are related to its nonselective inhibition of cyclooxygenase enzymes (COX), resulting in decreased production of prostaglandins, mediators of inflammation, pain (low to moderate), and fever.[1] In normal doses, acetaminophen is safe and effective, but when consumed in overdose quantities it may cause severe liver and, less frequently, kidney damage, or death.[1] The patient may have mild or no symptoms early after acute overdose of acetaminophen. Other than what can be found in the patient's history, the only reliable early diagnostic indicator is provided by a quantitative measurement of the serum acetaminophen level.[2] The prophylactic antidote, N-acetylcysteine, is highly effective in preventing liver damage if administered 8 to 10 hours after acetaminophen ingestion; if administered 12 to 16 hours after ingestion it is less effective, but should still be given because it improves survival in patients with hepatic failure.[2] Clinical evidence of liver and kidney damage is usually delayed for 24 hours or more after ingestion, well after the time that N-acetylcysteine can be effectively administered.[2] Therefore, assessment of serum acetaminophen concentration is one of the key elements to ensure appropriate and timely overdose management.[3,4,5]

Test principle

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of acetaminophen in human serum or plasma. 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.

Reagents - working solutions

R1	Anti-acetaminophen antibody (sheep polyclonal), G6P, NAD, bovine serum albumin, preservatives and stabilizers
R3	Acetaminophen labeled with bacterial G6PDH, Tris buffer, preservatives, bovine serum albumin, and stabilizers

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.
Prevention:	
P261	Avoid breathing mist or vapours.
P273	Avoid release to the environment.
P280	Wear protective gloves.
Response:	
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Disposal:	
P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

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:	26 weeks

Do not freeze.

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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, or lithium heparinized plasma.

Sample collection tubes containing separating gel have not been verified for use.

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.

Stability:	24 hours capped at RT
	7 days capped at 2-8 °C
	6 months capped at -20 °C (± 5 °C)

Freeze only once.

Invert thawed specimens several times prior to testing.

Do not induce foaming of specimens.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

See "Order information" section

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)	415/340 nm		
Reagent pipetting			
R1	70 µL	–	
R3	35 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.4 µL	–	–
Decreased	1.4 µL	15 µL	60 µL
Increased	1.4 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: H_2O
	S2-S6: ACET2 calibrator, dilution by instrument
Calibrators (2-point calibration)	S2, S5: ACET2 calibrator, dilution by instrument
Calibration mode	Non-linear
Calibration frequency	2-point calibration - every 7 days on-board
	Full calibration - after reagent lot change - 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. The calibrator is prepared to contain a known quantity of acetaminophen in buffer.

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 26 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, mg/L).

Conversion factors:[6]	µg/mL x 6.62 = µmol/L
	µg/mL x 1.0 = mg/L

Limitations - interference

Criterion: Interference is defined as not significant when recovery observed is within ± 1 µg/mL (6.6 µmol/L) of initial value at an acetaminophen concentration of approximately 5 µg/mL (33.1 µmol/L) and recovery within ± 10 % of initial value at an acetaminophen concentration of approximately 30 µg/mL (199 µmol/L).

Icterus:[7] No significant interference up to an I index of 30 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 510 µmol/L or 30 mg/dL).

Hemolysis:[7] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 496 µmol/L or 800 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 400. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from triglycerides from Intralipid up to 650 mg/dL if the L-index is below 400.

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[8]

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

5-200 µg/mL (33-1324 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

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Limits and ranges

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 1.5 µg/mL (9.9 µmol/L)
Limit of Detection	= 3 µg/mL (20 µmol/L)
Limit of Quantitation	= 5 µg/mL (33 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 acetaminophen samples.

Expected values

Normal therapeutic doses of acetaminophen result in serum concentrations of 10-30 µg/mL (66-199 µmol/L*) in healthy adults.[9]

The concentration of acetaminophen in serum or plasma depends on the time of drug ingestion; 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.

In acute acetaminophen overdose, a single serum or plasma level determination, plotted on the Rumack-Matthew nomogram,[10,11] provides a good indication of whether overdose therapy is required.[2]

Alcoholics are at risk for toxicity at lower doses. Enhanced susceptibility to toxic effects has also been reported in persons receiving long-term anticonvulsant therapy and patients taking isoniazid.[2]

Toxic manifestations have been observed at serum concentrations > 100 µg/mL (> 662 µmol/L*), however the toxic range is generally reported at > 200 µg/mL (> 1324 µmol/L*). Toxic concentrations can be more effectively related to post dose interval; > 200, > 100, and > 50 µg/mL (> 1324, > 662, and > 331 µmol/L*) serum concentrations correspond to toxic concentrations at 4, 8, and 12 hours post dose, respectively.[11]

* calculated by unit conversion factor

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

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.

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*	*SD*	*CV*
	µg/mL	*µg/mL*	*%*
TDMC1[a)]	13.6	0.195	1.4
TDMC2[b)]	36.5	0.358	1.0
TDMC3[c)]	110	1.52	1.4
Human serum 1	7.25	0.141	1.9
Human serum 2	21.0	0.289	1.4
Human serum 3	63.7	0.701	1.1
Human serum 4	99.5	1.27	1.3
Human serum 5	170	3.16	1.9

Intermediate precision	*Mean*	*SD*	*CV*
	µg/mL	*µg/mL*	*%*
TDMC1[a)]	13.6	0.434	3.2
TDMC2[b)]	36.5	1.09	3.0
TDMC3[c)]	105	3.48	3.3
Human serum 1	7.06	0.286	4.1
Human serum 2	21.0	0.615	2.9
Human serum 3	64.4	1.81	2.8
Human serum 4	104	3.06	2.9
Human serum 5	170	7.12	4.2

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) and **cobas c** 703 analyzer(s).

Method comparison

Acetaminophen values for human serum 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) = 75

Passing/Bablok[12]	Linear regression
y = 1.014x + 0.417 µg/mL	y = 1.014x + 0.625 µg/mL
τ = 0.989	r = 0.999

The sample concentrations were between 5.49 and 200 µg/mL.

Acetaminophen values for human serum 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) = 74

Passing/Bablok[12]	Linear regression
y = 1.030x + 1.30 µg/mL	y = 0.997x + 2.50 µg/mL
τ = 0.991	r = 0.999

The sample concentrations were between 5.16 and 199 µg/mL.

Acetaminophen values for human serum samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 72

Passing/Bablok[12]	Linear regression
y = 0.996x + 0.161 µg/mL	y = 1.004x - 0.0294 µg/mL
τ = 0.989	r = 0.999

The sample concentrations were between 6.27 and 197 µg/mL.

Analytical specificity

The following compounds were tested for cross-reactivity:

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Compound	Compound Concentration [µg/mL]	Concentration Acet-aminophen [µg/mL]	% Cross-reactivity
Acetaminophen cysteine	100	6.1	0.5
Acetaminophen glucuronide	1000	5.2	ND
Acetaminophen mercapturate	300	5.4	0.2
Acetaminophen sulfate	200	6.1	ND
Cysteine	1300	5.8	ND
N-Acetylcysteine	1663	6.3	ND
Phenacetin	500	6.7	0.5

Compound	Compound Concentration [µg/mL]	Concentration Acet-aminophen [µg/mL]	% Cross-reactivity
Acetaminophen cysteine	100	29.2	-0.3
Acetaminophen glucuronide	1000	25.4	-0.1
Acetaminophen mercapturate	300	25.9	0.2
Acetaminophen sulfate	200	27.8	0.1
Cysteine	1300	29.0	ND
N-Acetylcysteine	1663	28.5	ND
Phenacetin	500	29.3	1.3

ND = Not detectable

The following 24 drugs were tested for interference. No significant interference with the assay was found.

Acetyl cysteine	Phenylbutazone
Acetylsalicylic acid	Rifampicin
Ampicillin-sodium	Theophylline
Ascorbic acid	Amitriptylline
Cefoxitin	Caffeine
Cyclosporine	Codeine
Doxycycline	Diazepam
Heparin	Methionine
Ibuprofen	Phenylephrine
Levodopa	Propoxyphene
Methyldopa + 1.5 H_2O	Salicylate
Metronidazole	Secobarbital

References

1 Langman LJ, Bechtel LK, Holstege CP. Clinical Toxicology. In: Rifay N, Chiu RWK, Young I, Burnham CAD, Wittwer CT, editors. Tietz Textbook of Laboratory Medicine, Saunders Elsevier, Philadelphia, 7th edition, 2023, chapter 43, p. 454-454.e84.

2 Dale DC. ACP Medicine, 3rd edition. BC Decker Inc. 2007:161-162.

3 Wallace CI, Dargan PI, Jones AL. Paracetamol overdose: an evidence based flowchart to guide management. Emerg Med J 2002 May;19(3):202-205. Erratum in: Emerg Med J 2002 Jul;19(4):376.

4 Chiew AL, Reith D, Pomerleau A, et al. Updated guidelines for the management of paracetamol poisoning in Australia and New Zealand. Med J Aust 2020 Mar;212(4):175-183.

5 Chiew AL, Buckley NA. Acetaminophen Poisoning. Crit Care Clin 2021 Jul;37(3):543-561.

6 Tietz NW. Fundamentals of Clinical Chemistry, 6th ed. Saunders Elsevier 2008.

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

8 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

9 Jacobs DS, De Mott WR, Oxley DK. Laboratory Test Handbook with Key Word Index 5th ed. Hudson, Ohio:Lexi-Comp, Inc 2001:778-779.

10 Rumack BH, Matthew H. Acetaminophen Poisoning and Toxicity. Pediatrics 1975 Jun;55(6):871-876.

11 Rumack BH. Acetaminophen overdose. Arch Intern Med 1981;141:380-385.

12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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 (for USA: see navifyportal.roche.com for definition of symbols used):

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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