ACET2

ONLINE TDM Acetaminophen Gen.2

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REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
06769942 190	ONLINE TDM Acetaminophen Gen.2 (150 tests)	System-ID 07 7551 7	Roche/Hitachi **cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

07007515 190	ACET2 calibrator ACETC (1 x 2 mL)	Code 670	
04521536 190	TDM Control Set Level I (2 x 5 mL) TDM Control Set Level II (2 x 5 mL) TDM Control Set Level III (2 x 5 mL)	Code 310 Code 311 Code 312	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

ACET2: ACN 172

For **cobas c** 502 analyzers:

ACET2: ACN 8172

Intended use

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

Summary

Acetaminophen is a widely used analgesic and antipyretic found in a number of over-the-counter and prescription products. When consumed in overdose quantities, acetaminophen may cause severe liver and kidney damage, or death.[1]

The patient may have few or no symptoms early after acute overdose of acetaminophen. The only reliable early diagnostic indicator is provided by a quantitative measurement of the serum acetaminophen level. Clinical evidence of liver and kidney damage is usually delayed for 24 hours or more after ingestion, well after the time that the prophylactic antidote, acetylcysteine, can be effectively administered.[1] Acetylcysteine is highly effective in preventing liver damage, especially if administered within 8 to 10 hours after overdose, and improves survival in patients with hepatic failure when initiated 12 to 16 hours after overdose.[1]

The methods historically used to monitor serum acetaminophen concentrations are high-performance liquid chromatography, gas-liquid chromatography, UV spectrophotometry, and colorimetric immunoassay.[2]

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

R2 Acetaminophen labeled with bacterial G6PDH, Tris buffer, preservatives, bovine serum albumin, and stabilizers

R1 is in position A and R2 is in position C. Position B contains H_2O for technical reasons.

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

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 dust/fume/gas/mist/vapours/spray.
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, USA: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

ACET2

Shelf life at 2-8° C:	See expiration date on **cobas c** pack label
Do not freeze.	
On-board in use and refrigerated on the analyzer:	12 weeks

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

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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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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

Do not induce foaming of specimens. Specimens can be frozen and thawed up to 1 time.

Invert thawed specimens several times prior to testing.

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

cobas c 311 test definition

Assay type	Rate-A
Reaction time / Assay points	10 / 14-26
Wavelength (sub/main)	415 / 340 nm
Reaction direction	Increase
Units	µg/mL (µmol/L)
Reagent pipetting	Diluent (H_2O)
R1	100 µL	–
R2	50 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	2 µL	30 µL	120 µL
Increased	2 µL	–	–

cobas c 501/502 test definition

Assay type	Rate-A
Reaction time / Assay points	10 / 21-39
Wavelength (sub/main)	415 / 340 nm
Reaction direction	Increase
Units	µg/mL (µmol/L)
Reagent pipetting	Diluent (H_2O)
R1	100 µL	–
R2	50 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2 µL	–	–
Decreased	2 µL	30 µL	120 µL
Increased	2 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2-S6: ACET2 calibrator, dilution by instrument
Calibration mode	RCM
Calibration frequency	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. 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
Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factor: µg/mL x 6.62 = µmol/L[3]

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 level of approximately 5 µg/mL (33.1 µmol/L) and recovery within ± 10 % of initial value at an acetaminophen level of approximately 30 µg/mL (199 µmol/L).

Serum/Plasma

Icterus:[4] 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:[4] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 496 µmol/L or 800 mg/dL).

Lipemia (Intralipid):[4] 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.[5]

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

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Measuring range

5-200 µg/mL (33.1-1324 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.

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 and Limit of Detection 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 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.[2]

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[6,7], provides a good indication of whether overdose therapy is required.[1]

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.[1]

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.[7]

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 a Roche/Hitachi **cobas c** analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5-A2 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer:

Serum/Plasma

Repeatability	Mean		SD		CV
	µg/mL	µmol/L	µg/mL	µmol/L	%
Control 1	15.3	101	0.4	3	2.5
Control 2	34.9	231	0.9	6	2.5
Control 3	106	700	2	15	2.2
HS 1	7.7	50.9	0.2	1	2.9
HS 2	73.2	485	1.7	11	2.3

Repeatability	Mean		SD		CV
	µg/mL	µmol/L	µg/mL	µmol/L	%
HS 3	130	859	4	23	2.7
HS 4	168	1115	4	28	2.5
HS 5	184	1221	4	28	2.3

Intermediate precision	Mean		SD		CV
	µg/mL	µmol/L	µg/mL	µmol/L	%
Control 1	15.3	101	0.5	3	3.2
Control 2	34.9	231	1.0	7	2.8
Control 3	106	700	3	21	3.0
HS 1	7.4	48.9	0.3	2	3.5
HS 2	73.2	485	1.9	13	2.7
HS 3	130	859	4	28	3.2
HS 4	168	1115	5	35	3.2
HS 5	185	1225	6	36	3.0

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison
Serum/plasma

Acetaminophen values for human serum samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the Emit® tox™ Acetaminophen assay on Olympus AU5400 analyzer (x).

Sample size (n) = 105

Deming Regression Weighted[8]

y = 1.02x - 0.699 µg/mL

r = 0.997

The sample concentrations were between 5.2 and 198 µg/mL (34.4 and 1310 µmol/L).

Acetaminophen values for human serum samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using LC-MS/MS (x).[9]

Sample size (n) = 105

Deming Regression Weighted[8]

y = 0.984x - 0.116 µg/mL

r = 0.996

The sample concentrations were between 5.2 and 198 µg/mL (34.4 and 1310 µmol/L).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Analytical specificity

The following compounds were tested for cross-reactivity:

Compound	Compound Concentration [µg/mL]	Concentration Acet-aminophen [µg/mL]	% Cross-reactivity
Acetaminophen cysteine	100	6.1	0.5
Acetaminophen glucuronide	1000	5.2	n.d.*
Acetaminophen mercapturate	300	5.4	0.2

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Compound	Compound Concentration [µg/mL]	Concentration Acet- aminophen [µg/mL]	% Cross- reactivity
Acetaminophen sulfate	200	6.1	n.d.*
Cysteine	1300	5.8	n.d.*
N-Acetylcysteine	1663	6.3	n.d.*
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	n.d.*
N-Acetylcysteine	1663	28.5	n.d.*
Phenacetin	500	29.3	1.3

* n.d. = 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   Dale DC. ACP Medicine, 3rd edition. BC Decker Inc. 2007:161-162.

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

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

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

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

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

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

8   Linnet, K. Evaluation of regression procedures for method comparison studies. Clinical Chemistry 1993 Mar;39(3):424-432.

9   Bylda C, Thiele R, Kobold U, et al. Simultaneous quantification of acetaminophen and structurally related compounds in human serum and plasma. Drug Test Anal 2014 May;6(5):451-460 (JCTLM C11RMP8).

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

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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