ACET2

ONLINE TDM Acetaminophen Gen.2

cobas®
Therapeutic drug monitoring

Order information

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	COBAS INTEGRA 400 plus
Materials required (but not provided):			
07007515 190	ACET2 calibrator (1 × 2 mL)	System-ID 07 7564 9	
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	
20756350 322	NaCl Diluent 9 %	System-ID 07 5635 0	

English

System information

Test ACET2, test ID 0-040.

Intended use

In vitro diagnostic test for the quantitative determination of acetaminophen overdose in serum and plasma on COBAS INTEGRA 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
SR	Acetaminophen labeled with bacterial G6PDH, Tris buffer, preservatives, bovine serum albumin, and stabilizers

R1 is in position A and SR 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

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
Do not freeze.	
On board in use at 10-15 °C	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 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 room temperature

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.

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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board the COBAS INTEGRA 400 plus analyzer.

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

Test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	340/409 nm
Calc. first/last	42/59
Unit	µg/mL

Pipetting parameters

		Diluent (H_2O)
R1	100 µL	
SR	50 µL	
Sample	3 µL	5 µL
Total volume	158 µL	

Calibration

Calibrator	ACET2 calibrator, dilution performed automatically by instrument Enter the assigned ACET2 calibrator values for all 6 calibrator points stated in the ACET2 Calibrator Value Sheet.
Calibration mode	logit/log 4
Calibration replicate	Duplicate recommended
Calibration interval	Each lot change, every 4 weeks, and 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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: µg/mL × 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.

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 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

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.

Lower limits of measurement

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 the COBAS INTEGRA analyzers 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-A3 requirements. The following results were obtained on a COBAS INTEGRA 400 plus analyzer with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days):

Repeatability	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Control 1	15.8 (105)	0.6 (4)	3.8
Control 2	34.2 (226)	1.2 (8)	3.4
Control 3	107 (708)	4 (28)	4.0
HS 1	7.6 (50.0)	0.4 (2.6)	5.1
HS 2	77.1 (510)	2.6 (17)	3.3
HS 3	139 (920)	6 (39)	4.2
HS 4	184 (1218)	7 (48)	3.9
HS 5	193 (1278)	7 (47)	3.7

Intermediate precision	*Mean µg/mL (µmol/L)*	*SD µg/mL (µmol/L)*	*CV %*
Control 1	15.8 (105)	1.0 (7)	6.5
Control 2	35.5 (235)	2.4 (16)	6.6
Control 3	111 (735)	8 (50)	6.8
HS 1	7.6 (50.0)	0.5 (3.1)	6.1
HS 2	78.1 (517)	4.7 (31)	6.0
HS 3	138 (914)	8 (53)	5.8
HS 4	185 (1225)	13 (85)	7.0
HS 5	193 (1278)	12 (77)	6.0

Method comparison

Acetaminophen values for human serum samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined with the Emit® tox™ Acetaminophen assay on Olympus AU5400 analyzer (x).

Sample size (n) = 106

Deming Regression Weighted[8]

y = 1.01x - 0.957 µg/mL

r = 0.993

The sample concentrations were between 5.1 and 198 µg/mL (34.0 and 1311 µmol/L).

Acetaminophen values for human serum samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined with LC-MS/MS (x).[9]

Sample size (n) = 106

Deming Regression Weighted[8]

y = 0.973x - 0.380 µg/mL

r = 0.993

The sample concentrations were between 5.1 and 198 µg/mL (34.0 and 1311 µmol/L).

Analytical specificity

The following compounds were tested for cross-reactivity:

Compound	Compound Conc. [µg/mL]	Conc. Acetaminophen [µg/mL]	Cross-reactivity %
Acetaminophen cysteine	100	7.1	0.4
Acetaminphen glucuronide	1000	5.8	ND
Acetaminophen mercapturate	300	5.5	0.1
Acetaminophen sulfate	200	6.7	ND
Cysteine	1300	6.4	ND
N-Acetylcysteine	1663	6.4	ND
Phenacetin	500	7.2	0.6

Compound	Compound Conc. [µg/mL]	Conc. Acetaminophen [µg/mL]	Cross-reactivity %
Acetaminophen cysteine	100	29.6	1.0
Acetaminphen glucuronide	1000	26.0	ND
Acetaminophen mercapturate	300	25.1	0.2
Acetaminophen sulfate	200	29.9	0.2
Cysteine	1300	29.0	0.1
N-Acetylcysteine	1663	30.3	ND
Phenacetin	500	32.4	1.4

ND = not detectable

Tests were performed on 24 drugs. No significant interference with the assay was found.

Acetyl cysteine Phenylbutazone

Acetylsalicylic acid	Rifampicin
Ampicillin-sodium	Theophylline
Ascorbic acid	Amitriptyline
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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Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Distribution in USA by:
Roche Diagnostics, Indianapolis, IN
US Customer Technical Support 1-800-428-2336