ACETA

cobas®

Acetaminophen

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20767174 322	Acetaminophen 150 tests	System-ID 07 6717 4	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
20758809 122	COBAS Acetaminophen Calibrators CAL A-B (2 x 3 mL)	Codes 686-687	
04521536 190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 310 Code 311 Code 312	

English

System information

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

ACETA: ACN 414

For **cobas c** 502 analyzers:

ACETA: ACN 8414

Intended use

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

Summary

Acetaminophen is a common drug used in many formulations due to its analgesic and antipyretic properties.[1] Chronic excessive use of acetaminophen can result in hepatotoxicity and nephrotoxicity.[2,3] Overdosage can lead to severe hepatic damage and hepatic failure if untreated.[4,5,6] Early diagnosis of acetaminophen induced hepatotoxicity is important since initiation of therapy within 16 hours of ingestion lessens the potential for hepatic injury and decreases the mortality rate.[7] Therefore, a rapid and accurate determination of acetaminophen is needed.

Test principle

Acetaminophen is hydrolyzed by an arylacylamidase to yield p-aminophenol and acetate. Subsequently the p-aminophenol is converted to an indophenol in the presence of o-cresol and a periodate catalyst. The production of indophenol is followed colorimetrically. The change in absorbance is directly proportional to the quantitative drug concentration in the sample.

Reagents - working solutions

R1 Sodium periodate 3.75 mmol/L

R2 Arylacylamidase (microbial) ≥ 7000 U/L; o-cresol 3.75 mmol/L

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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 and refrigerated on the analyzer:	21 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.
Nonhemolyzed serum: Collect serum using standard sampling tubes.
Nonhemolyzed plasma: Li-heparin plasma, K_2-EDTA and K_3-EDTA plasma.

Stability:[8]	48 hours capped at 2-8 °C
	4 weeks capped at -20 °C

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.

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)

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

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

cobas c 311 **test definition**

Assay type	2-Point End		
Reaction time /Assay points	10 / 21-48		
Wavelength (sub/main)	800 /600 nm		
Reaction direction	Increase		
Unit	µg/mL		
Reagent pipetting		Diluent (H_2O)	
R1	50 µL	20 µL	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End	
Reaction time /Assay points	10 / 31-60	
Wavelength (sub/main)	800 /600 nm	
Reaction direction	Increase	
Unit	µg/mL	
Reagent pipetting		Diluent (H_2O)

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R1	50 µL	20 µL	
R2	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

The technical limit in the instrument setting is defined as -2.7-496.1 µg/mL due to the instrument factor of +3.9.

Calibration

Calibrators	S1-2: COBAS Acetaminophen Calibrators
Calibration mode	Linear
Calibration frequency	2-point calibration • after **cobas c** pack change • as required following quality control procedures

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities 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[9]

ACTION REQUIRED

A correction value +3.9 µg/mL (25.8 µmol/L) is required for this acetaminophen procedure. Enter the correction value as the instrument factor y = ax + b for µg/mL or for µmol/L, where a = 1.0 and b = 3.9 (µg/mL) or a = 1.0 and b = 25.8 (µmol/L).

Limitations - interference

Icterus, Hemolysis, Lipemia:
The serum index cutoff values in the application settings are based on the acetaminophen concentration of 50 µg/mL (331 µmol/L) and should be adjusted to the intended use of the assay as appropriate.

Criterion: Recovery within ± 1 µg/mL (6.6 µmol/L) of initial value at an acetaminophen level of approximately **5 µg/mL** (33.1 µmol/L).
Icterus:[10] Interference occurs with icteric samples (I Index > 1, approximate bilirubin concentration of 1.0 mg/dL (17 µmol/L)).
Hemolysis:[10] Interference occurs with hemolytic samples (H Index > 10, approximate hemoglobin concentration of 10 mg/dL (6.2 µmol/L)).
Lipemia (Intralipid):[10] Interference with lipemic samples (L Index > 100). There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at an acetaminophen level of approximately **30 µg/mL** (199 µmol/L).
Icterus:[10] No significant interference up to an I index of 14 (approximate conjugated and unconjugated bilirubin concentration: 14 mg/dL (239 µmol/L)).
Hemolysis:[10] No significant interference up to an H index of 250 (approximate hemoglobin concentration: 250 mg/dL (155 µmol/L)).
Lipemia (Intralipid):[10] No significant interference up to an L Index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at an acetaminophen level of approximately **50 µg/mL** (331 µmol/L).
Icterus:[10] No significant interference up to an I index of 25 (approximate conjugated and unconjugated bilirubin concentration: 25 mg/dL (427 µmol/L)).
Hemolysis:[10] No significant interference up to an H index of 150 (approximate hemoglobin concentration: 150 mg/dL (93 µmol/L)).
Lipemia (Intralipid):[10] No significant interference up to an L index of 1200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other interferences:

Criterion: Recovery within ± 10 % of initial value at an acetaminophen level of approximately 50 µg/mL (331 µmol/L).

Total protein: No interference from total protein from 2.0 g/dL to 12 g/dL.

Amitriptyline and Imipramine showed a significant negative interference (≥ 10 %).

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.[11]

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/Multiclean/SCCS or 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 not required.

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

Limits and ranges

Measuring range

1.2-500 µg/mL (7.94-3310 µmol/L)

Manually dilute samples above the measuring range 1 + 2 with the 0 µg/mL calibrator and reassay. Multiply the result by 3 to obtain the specimen value.

Lower limits of measurement

Lower detection limit

1.2 µg/mL (7.94 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 21).

Expected values

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.[12] The therapeutic range varies and has been reported to be 10 to 30 µg/mL (66 to 199 µmol/L).[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[8]

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

Precision

Precision was determined using human samples and controls in a modified NCCLS EP5-T2 protocol (repeatability n = 63, intermediate precision n = 63). The following results were obtained on a Roche/Hitachi **cobas c** 501 analyzer.

Serum/Plasma

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Repeatability	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.9	39.1	0.27	1.8	4.5
Control 2	31.4	207.9	0.30	2.0	1.0
Control 3	104.6	692.4	0.74	4.9	0.7
HS 1	18.5	122.5	0.38	2.5	2.0
HS 2	99.6	659.4	0.55	3.6	0.6

Intermediate precision	*Mean*		*SD*		*CV*
	µg/mL	*µmol/L*	*µg/mL*	*µmol/L*	*%*
Control 1	5.9	39.1	0.41	2.7	6.9
Control 2	31.4	207.9	0.80	5.3	2.6
Control 3	104.6	692.4	2.62	17.3	2.5
HS 1	18.5	122.5	0.68	4.5	3.7
HS 2	99.6	659.4	2.34	15.5	2.4

Method comparison

Serum/plasma

Acetaminophen values for human serum and plasma samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with the corresponding reagent on COBAS INTEGRA 800 analyzer (x) and Roche/Hitachi 917 analyzer (x).

COBAS INTEGRA 800 analyzer	Sample size (n) = 115
Passing/Bablok[13]	Linear regression
y = 1.016x - 0.384 µg/mL	y = 1.014x - 0.375 µg/mL
τ = 0.973	r = 1.000

The sample concentrations were between 4.4 and 290 µg/mL (29 and 1920 µmol/L).

τ = Kendall's tau.

Roche/Hitachi 917 analyzer	Sample size (n) = 122
Passing/Bablok[13]	Linear regression
y = 1.028x - 0.323 µg/mL	y = 1.026x - 0.203 µg/mL
τ = 0.985	r = 1.000

The sample concentrations were between 4.3 and 481 µg/mL (28 and 3184 µmol/L).

τ = Kendall's tau.

Analytical specificity

The following compounds were tested for cross-reactivity.

Compound	**Concentration Tested (µg/mL)**	**% Cross-reactivity**
p-Phenetidine	137	24.5
N-acetylbenzoquinoneimine	300	20.4
Acetophenetidin	300	4.9
Amitriptyline-HCl	277	*
Amphetamine	135	*
Imipramine-HCl	280	*
Acetaminophen glucuronide	300	ND
4-Acetamidothiophenol	300	ND
Acetanilide	300	ND
Benzoic acid	1000	ND
Butalbital	100	ND
Caffeine	1000	ND
Chlorpheniramine	100	ND
Chlorpromazine-HCl	100	ND
Chlorzoxazone	500	ND
Cysteamine	500	ND
Dihydrocodeine	20	ND
Diphenhydramine-HCl	500	ND
Hydrocodone bitartrate	20	ND
Ibuprofen	500	ND
Indomethacin	500	ND
Methionine	500	ND
N-acetylcysteine	500	ND
Naprosyn	500	ND
Oxycodone	20	ND
Phenobarbital	400	ND
Phenylephrine	20	ND
Promethazine-HCl	500	ND
Propoxyphene	20	ND
Pseudoephedrine	20	ND
Salicylate	1000	ND
Salicylamide	1000	ND
Theophylline	300	ND

*Negative cross reactivity noted. Re-evaluated as interferent. Refer to the Limitations section of this insert.

ND = not detectable

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

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

References

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6 Meredith TJ, Vale JA. In: Poisoning; Diagnosis and Treatment 1981;104.

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

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12 Rumack BH. Acetaminophen overdose. Arch Intern Med 1981;141:380.

13 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.

General reference: Kociancic T, Reed M. Acetaminophen Intoxication and Length of Treatment: How Long is Long Enough? Pharmacotherapy 2003;23(8):1052-1059.

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

CE

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