

ACETA

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 COBAS INTEGRA 400 plus COBAS INTEGRA 800
20758809 122	COBAS Acetaminophen Calibrator Calibrators A-B (2 × 3 mL)	System-ID 07 5880 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

English

System information

Test ACETA, test ID 0-117

Intended use

In vitro diagnostic test for the quantitative determination of toxic levels of acetaminophen in serum or heparinized plasma on COBAS INTEGRA 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

Enzymatic method

COBAS INTEGRA systems therapeutic drug monitoring measurements are made on the COBAS INTEGRA systems using an enzymatic reaction. 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 serum.

Reagents - working solutions

- R1** Enzyme reagent
Arylacylamidase (microbial) ≥ 7000 U/L, o-cresol 3.75 mmol/L
- R2** Catalyst reagent
Sodium periodate 3.75 mmol/L

R1 is in position A and R2 is in position B.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 21 weeks

The on-board in use stability period begins at the time of **cobas c** pack puncture.

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.

Unhemolyzed serum

Unhemolyzed heparinized plasma

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 be tested within 8 hours of collection if kept at room temperature. If specimens must be stored for later testing, they may be kept at 2-8 °C for up to 48 hours or at -20 °C for 4 weeks.⁸ 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.

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

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S
Reaction direction	Increase
Wavelength A	629 nm
Calc. first/last	32/58
Unit	µg/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	50 µL	
R2	50 µL	
Sample	2.5 µL	20 µL
Total volume	122.5 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1/R2-S
Reaction direction	Increase
Wavelength A	629 nm
Calc. first/last	48/90
Unit	µg/mL

ACETA

Acetaminophen

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Therapeutic drug monitoring

Pipetting parameters

		Diluent (H ₂ O)
R1	50 µL	
R2	50 µL	
Sample	2.5 µL	20 µL
Total volume	122.5 µL	

The measuring range in the instrument settings is defined as 0-298 µg/mL due to the correlation offset of 2.

Calibration

Calibrator	COBAS Acetaminophen calibrator Calibrators A-B
Acetaminophen conc.	0, 300 µg/mL (0, 1986 µmol/L)
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	<i>COBAS INTEGRA 400 plus analyzer:</i> Each cobas c pack and 2 weeks <i>COBAS INTEGRA 800 analyzer:</i> Each cobas c pack and 3 weeks

A calibration curve must be prepared using the COBAS Acetaminophen calibrators. Calibrators must be placed from the higher concentration (B) first, to the lower (A) last, on the CAL/QC rack. This curve is retained in memory by the COBAS INTEGRA systems and recalled for later use.

Traceability: The COBAS Acetaminophen calibrators are prepared to contain known quantities of acetaminophen in normal human serum and are traceable to USP reference standards.

Note

Calibrators should be assayed within 2 hours after placing on-board the instrument.

Quality control

Quality control	TDM Control Set
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

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.

Note

Controls should be assayed within 2 hours after placing on-board the instrument.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: µg/mL × 6.62 = µmol/L⁹

Limitations - interference

See the Analytical specificity section of this method sheet for information on substances tested for cross-reactivity in this assay. There is the possibility that other substances and/or factors may interfere with the test and cause erroneous results (e.g., technical or procedural errors).

Specimens with assay values greater than 300 µg/mL (1986 µmol/L) will be flagged by the system and must be repeated after appropriate manual dilution of the original sample with the zero calibrator.

1. 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 17 µmol/L or 1.0 mg/dL).

Hemolysis:¹⁰ Interference occurs with hemolytic samples (H index > 10, approximate hemoglobin concentration of 6.2 µmol/L or 10 mg/dL).

Lipemia (Intralipid):¹⁰ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

2. 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 9 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 154 µmol/L or 9 mg/dL).

Hemolysis:¹⁰ No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):¹⁰ No significant interference up to an L index of 1800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

3. 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 12 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 205 µmol/L or 12 mg/dL).

Hemolysis:¹⁰ No significant interference up to an H index of 350 (approximate hemoglobin concentration: 217 µmol/L or 350 mg/dL).

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.

Total protein: No significant interference up to a total protein concentration of 14.4 g/dL^{a)}

Amitriptyline showed a significant negative interference (≥ 10 %) at 277 µg/mL.

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.

a) measured at an acetaminophen concentration of approximately 50 µg/mL (331 µmol/L)

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

2-300 µg/mL (13.2-1986 µmol/L)

Lower limits of measurement

Lower detection limit of the test:

2 µg/mL (13.2 µmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from the zero calibrator at a 95 % confidence level.

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 10-30 µg/mL (66-198 µmol/L).⁷

ACETA

Acetaminophen

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 controls in accordance with the NCCLS EP5-T2¹³ requirements with repeatability and intermediate precision (2 aliquots per run, 2 runs per day, 20 days). The following results were obtained on a COBAS INTEGRA 700 analyzer.

Repeatability	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	9.9 (66)	0.6 (4)	5.8
Level 2	32.9 (218)	0.3 (2)	0.9
Level 3	97.4 (645)	0.7 (5)	0.7

Intermediate precision	Mean µg/mL (µmol/L)	SD µg/mL (µmol/L)	CV %
Level 1	9.9 (66)	0.7 (5)	7.5
Level 2	32.9 (218)	1.5 (10)	4.4
Level 3	97.4 (645)	4.8 (32)	4.9

Method comparison

Acetaminophen values for human serum samples obtained on a COBAS INTEGRA 800 analyzer using the COBAS INTEGRA Acetaminophen reagent (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c** 501 analyzer (x). Sample size (n) = 115

Passing/Bablok¹⁴

y = 0.984x - 1.460 µg/mL

r = 0.973

Linear regression

y = 0.985x - 1.399 µg/mL

r = 1.00

The sample concentrations were between 6.4 and 292 µg/dL (45 and 1932 µmol/L).

Analytical specificity

The following cross-reactive, structurally related and/or co-administered substances were evaluated on the COBAS INTEGRA systems in normal human serum spiked with acetaminophen at 100 µg/mL (662 µmol/L). Each substance was tested at 10 times the highest concentration for its therapeutic or normal range, as per the protocol described by NCCLS.¹⁵ The imprecision of the assay was taken into account when determining cross-reactivity. Cross-reactivity was designated as "not detectable" (ND) if the obtained value was less than the sensitivity of the assay.

Cross-reactivity (%) = $\frac{100 \times (\text{analytical result} - \text{analyte concentration})}{\text{concentration of interferent}}$

Drug	Level tested µg/mL	Cross-reactivity %
Acetaminophen glucuronide	300	ND
4-Acetamidothiophenol	300	ND
Acetanilide	300	ND
Acetophenetidin	300	12.2
Amphetamine	135	ND
Benzoic acid	1000	ND
Caffeine	1000	ND
Chlorpheniramine	100	ND
Chlorpromazine	100	ND
Chlorzoxazone	500	ND
Cysteamine	500	ND

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Drug	Level tested µg/mL	Cross-reactivity %
Diphenhydramine	500	ND
Ibuprofen	500	ND
Imipramine	280	ND
Indomethacin	500	ND
Methionine	500	ND
N-Acetylbenzoquinoneimine	300	45.7
N-Acetylcysteine	500	ND
Naprosyn	500	ND
p-Phenetidine	137	31.1
Phenobarbital	400	ND
Promethazine	500	ND
Salicylate	1000	ND
Salicylamide	1000	0.1
Theophylline	300	0.6

ND = Not Detectable

Any modification of the instrument as set forth in this labeling requires validation by the laboratory.

References

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- 12 Rumack BH. Acetaminophen overdose. Arch Intern Med 1981;141:380.
- 13 National Committee for Clinical Laboratory Standards. User Evaluation of Precision Performance of Clinical Chemistry Devices; Tentative Guideline. Villanova, PA.: NCCLS;1992;4(12). NCCLS Publication EP5-T2.
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- 15 National Committee for Clinical Laboratory Standards. Interference Testing in Clinical Chemistry; Proposed Guideline. Villanova, PA.: NCCLS; 1986;6(13). NCCLS Publication EP7-P.

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.

ACETA

Acetaminophen

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Therapeutic drug monitoring

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

GTIN

Global Trade Item Number

COBAS, COBAS C and COBAS INTEGRA are trademarks of Roche.

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Significant additions or changes are indicated by a change bar in the margin.

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