# ACET2

cobas®

**ONLINE TDM Acetaminophen Gen.2**

| 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 |
| **07007515** 190 | ACET2 calibrator ACETC (1 x 2 mL) | Code 670 | |
| **04521536** 190 | TDM Control Set Level I (2 x 5 mL)<br>TDM Control Set Level II (2 x 5 mL)<br>TDM Control Set Level III (2 x 5 mL) | Code 310<br>Code 311<br>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.
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.

For USA: For prescription use only.

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

Warning

| | |
|---|---|
| H317 | May cause an allergic skin reaction. |

**Prevention:**

| | |
|---|---|
| P261 | Avoid breathing dust/fume/gas/mist/vapours/spray. |
| 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. |

Product safety labeling primarily 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.

Serum 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.

| | |
|---|---|
| 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

# ACET2

**ONLINE TDM Acetaminophen Gen.2**

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<br>- after reagent lot change<br>- as required following quality control procedures |

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 not required.

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

**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 95$^{th}$ 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 |
| 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 |

### Method comparison

**Serum/plasma**

Acetaminophen values for human serum samples obtained on Roche/Hitachi **cobas c** 501 analyzer (y) were compared to those determined with 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 to those determined with LC/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).

### 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 |
| 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
Acetylsalicylic acid
Phenylbutazone
Rifampicin

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| | |
|---|---|
| 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.

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.

**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 |

**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.




Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

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