



PARACETAMOL (Acetaminophen) Assay Kit



Instructions for Use



For *in vitro* diagnostic use only



Store in DARK at 2 to 8°C

DO NOT FREEZE

REF	K8001	K8002
Lyophilised Enzyme	1 vial	3 vials
Enzyme Diluent	1 x 45mL	1 x 45mL
Colour Reagent A	1 x 65mL	1 x 65mL
Colour Reagent B	1 x 65mL	1 x 65mL
Aqueous Calibrator (standard) (2.0 mmol/L / 302 mg/L)	1 x 3.0mL	2 x 3.0mL



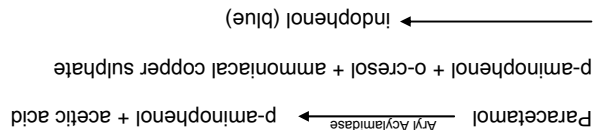
Cambridge Life Sciences Ltd
14 St. Thomas' Place
Cambridgeshire Business Park
Ely, Cambs. CB7 4EX UK
Tel: +44(0)1353 645200 Fax: +44(0)1353 645250
email: sales@clsdiagnostics.com
www.clsdiagnostics.com

1. Clinical Application

Paracetamol is a commonly used analgesic which, if taken in excessive amounts, can lead to toxic liver damage and, less commonly, renal impairment.¹⁻³ The major metabolites of paracetamol are the glucuronide and sulphate derivatives. A small proportion of a metabolite formed by microsomal oxidation is conjugated to glutathione and excreted subsequently as cysteine or mercapturate conjugates. If the glutathione stores of the liver become depleted in the presence of a large amount of paracetamol, the oxidised metabolite combines with liver cell components causing hepatic necrosis.⁴⁻⁵ The hepatocellular damage can be reduced by giving the patient compounds containing thiol groups such as methionine and N-acetyl cysteine.⁶⁻¹² The need to give one of these compounds is assessed on the measurement of the concentration of the parent drug⁷⁻⁸ in the blood, between four and twelve hours after ingestion.

2. Principle of the Assay

The method is based on the use of an enzyme specific for the amide bond of acetylated aromatic amines. It cleaves the paracetamol molecule, yielding p-aminophenol, which reacts specifically with o-cresol in ammoniacal copper solution to produce a blue colour.^{9,10,11} The assay is specific for the parent compound and does not detect paracetamol metabolites.



3. Sample

Human serum or plasma are the recommended samples. For serum, ensure complete clot formation prior to centrifugation. For both serum and plasma, separate the red blood cells or gel as soon after collection as possible. Acceptable anticoagulants are heparin, EDTA, fluoride oxalate and citrate.

Assay Procedure

It is recommended that samples be tested in duplicate. Experience with the technique may, however, suggest that single determinations of samples are adequate at the discretion of the user. A paracetamol calibrator (standard) is included with the reagents and should be used to calibrate the manual procedure with each run.

Equipment and Reagents not Supplied

Spectrophotometer capable of reading to 1.5 absorbance units at 615nm, with 1cm light path cells or an equivalent flow through cuvette. 50µL, 0.5mL, 1.0mL and 10mL pipettes. Distilled or deionised water.

Reagent Blank

The reagent blank should be constant for each kit. To measure it, use distilled water as the sample and follow the assay procedure.

4. Use of the Kit

Reconstitute each vial of lyophilised enzyme when required. Remove the cap and stopper, add 10mL of enzyme diluent. Replace the stopper and swirl contents, inverting occasionally. Allow to stand at room temperature for five minutes. After reconstitution the enzyme reagents is stable for 3 months or until the expiry date, whichever is sooner, if stored at 2 to 8°C.

If the lyophilised enzyme appears as a small hard pellet or is difficult to dissolve then the reagent should not be used.

It should be noted that patient samples containing conjugates of paracetamol, which have been stored for more than two weeks at room temperature, may yield paracetamol as a result of degradation of the conjugates.

6. References

- Ambre, J and Alexander, M; Liver toxicity after acetaminophen ingestion. J. Am. Med. Assoc. 238, 500-501 (1977).
- Black, M; Acetaminophen hepatotoxicity. Gastroenterol. 78, 382-392 (1980).
- Prescott, L F; The nephrotoxicity and hepatotoxicity of antipyretic analgesics. Brit. J. Clin. Pharmacol. 7, 443-452 (1979).
- Davis, M, Labadarios, D and Williams, R S; Metabolism of paracetamol after therapeutic and hepatotoxic doses in man. J. Int. Med. Res. 4, 40-45 (1976).
- Mitchell, J R, Jollow, D J, Potter, W Z, Davies, D C, Gillette, J R, Brodie, B B; Acetaminophen-induced hepatic necrosis. 1. Role of drug metabolism. J. Pharmacol. Exp. Therap. 187, 185-194 (1973).
- Meredith, T J Volans, G N; Paracetamol toxicity and its treatment. Recent Advances in Clinical Pharmacology 2, Edinburgh, Churchill Livingstone. 130-148 (1980).
- Meredith, T J and Vale, J A; Paracetamol poisoning. Poisoning Diagnosis and Treatment, London, Update Books, 104-112 (1981).
- Prescott, L F; Prevention of hepatic necrosis following paracetamol overdosage. Health Bulletin 4, 204-212 (1979).
- Hammond, P M, Price, C P and Scawen, M D; Production of aryl acylamidase. US Patent Number US4430433 (1984).
- Atkinson, A, Hammond, P M, Price, C P and Scawen, M D; Method for the estimation of N-acylated primary aromatic amines. US Patent Number US4414327(1983).
- Price, C P, Hammond P M and Scawen, M D; Evaluation of an enzymic procedure for the measurement of acetaminophen. Clin. Chem. 29, 358-361 (1983).
- Prescott, L F, Donavan, J W, Jarvie, D R, Proudfoot, A T; The disposition and kinetics of intravenous n-acetyl cysteine in patients with paracetamol overdosage. Eur. J. Clin. Pharmacol. 37: 501-506 (1989)
- Burtis, CA, Ashwood, ER editors; Tietz Textbook of Clinical Chemistry, 2nd Ed. Philadelphia, PA: WB Saunders; 1994:2212

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- For each assay dispense 50µL of either sample, calibrator or control into the respective cuvettes.
- Add 0.5mL of reconstituted enzyme reagent and mix well.
- Incubate at room temperature (15 to 25°C) for five minutes.
- To each cuvette add 1mL of colour reagent A. Then add 1mL of colour reagent B and mix well.
- Incubate for a minimum of four minutes and a maximum of sixty minutes at room temperature (15 to 25°C). Read absorbances at 615nm using a spectrophotometer.

Calculation of Results

Sample concentration (mmol/L) = (Sample absorbance - B) x S

(Calibrator absorbance - B)

Where B = blank absorbance, S = calibrator concentration (2.0mmol/L or 302mg/L).

Conversion: mmol/L x 151 = mg/L, mg/L / 151 = mmol/L

These instructions describe how to perform the assay manually with a spectrophotometer. However, it is also possible to run this assay on a range of automated analysers. Protocols are available for most clinical chemistry automates. The general parameters and the conditions have been validated by Cambridge Life Sciences. For further information, please contact Customer Services at Cambridge Life Sciences or your local distributor. Current versions of the protocols can be obtained from our website: www.clsdiagnostics.com and it is advised that the user confirms that the current version is being used.

Quality Control

Good laboratory practice requires that quality control specimens be included in every run to monitor assay performance. The quality control samples should be assayed repeatedly to establish mean values and working ranges.

The absorbance of the 2.0mmol/L (302mg/L) calibrator should be between 0.80 and 1.05 after subtraction of the blank absorbance.

Therapeutic Range

The therapeutic concentrations vary significantly depending on the individual patient. A range of 66-199µmol/L (10-30mg/L) may be an effective sample concentration in many patients¹³.

Accuracy

When patient serum samples were assayed and the results compared with those obtained by using an HPLC method, the following regression equation resulted:

$$y = 1.04x + 0.005 \text{ (mmol/L)}, r = 0.996, n = 61$$

where y = kit method and x = HPLC.

(Values shown are for an automated analysis).

Sensitivity

The sensitivity, defined as two standard deviations from zero (blank subtracted), was found to be 0.01 mmol/L (1.51mg/L).

Linearity

The method is linear up to a sample paracetamol concentration of 2.5 mmol/L (378mg/L).

Recovery

Analysis of serum samples to which known amounts of paracetamol had been added gave a mean recovery of 100.1% (range 98.6 to 103.1%).

Non-specific Absorbance

Paracetamol is not a physiological analyte. Samples of normal (paracetamol free) plasma or serum will yield a small absorbance, typically less than 0.04 after blank subtraction.

Interferences

No interference was found using heparin, EDTA, fluoride oxalate and citrate blood collection tubes. The addition of Bilirubin at 250mg/L, Haemoglobin at 4g/L or Intralipid at 2.5g/L to paracetamol samples results in no significant change to the paracetamol concentration recorded. Lipids above 2.5g/L may be removed using Lipoclear tubes taking into account the 20% dilution effect.

If the patient sample is highly pigmented or lipaemic, it is recommended that a sample blank is set up. Replace the enzyme reagent with distilled water and follow the assay procedure.

Safety Precautions

For *in-vitro* diagnostic use only.

For Professional use only.

Colour Reagent A contains 0.9% o-cresol. Avoid contact with skin and eyes. This is harmful in contact with skin and if swallowed. It is also irritating to eyes. In case of contact with skin, wash immediately with plenty of water. If you feel unwell, seek medical advice immediately. Colour Reagent B is irritating to eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Used samples, controls and pipette tips should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves be worn when handling such items. Safety data sheets are available upon request.

5. Performance

Precision

Typical precision for the assay is as follows:

C.V. (%)	Intra assay	Inter assay
30.2	0.005	0.012
0.755	0.004	0.012
2.4	0.604	1.812
1.1	0.804	1.17
1.0	1.812	1.17
2.6	0.906	0.23
1.5	0.906	0.41
1.7	3.02	1.18

This data was obtained from plasma, using manual pipettes and with twenty determinations in each case.

Accuracy

The method does not measure the common metabolites of paracetamol (glucuronide, sulphate, cysteine and mercapturate). In addition, no reaction was obtained with the following drugs at a concentration of 1 mmol/L:

n-acetyl cysteine†	methaqualone
acetylsalicylic acid†	nitrazepam*
amylodobarbitone	oxypertine
amtrypylline	p-aminosalicylic acid
amphetamine	pentazocine
caffeine	p-ethoxyacetanilide(phenacetin)
chloridiazepoxide*	p-ethoxyaniline (phenetidn)
chlomezazone	phenobarbitone
chlorpropamide	phenytoin
dextropropoxyphene	promethazine
diazepam*	salicylamide
dihydrocodeine	salicylic acid†
diphenhydramine	salicylic acid
imipramine	secobarbitone
indomethacin	sodium barbitione
lorazepam*	theophylline
meprobamate	tolbutamide
methadone	

* Tested at recorded peak plasma overdose concentrations (less than 1 mmol/L).

† Tested at a concentration of 5 mmol/L.

‡ Tested at a concentration of 1 g/L.

Important: Although the use of the manual method does not indicate any significant interference (under recovery) in the presence of n-acetyl cysteine, users of this product with automated analysers should follow the Cambridge Life Sciences protocols to avoid any possible interference. Any changes to these protocols should be verified by the user.

Calibrator Standardisation

Paracetamol calibrators are manufactured using primary calibration material, Acetaminophen (98.0% - 101.0%) that meets USP specifications. They are manufactured gravimetrically and tested against independent controls.