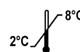
PARACETAMOL

(Acetaminophen) Assay Kit

Instructions for Use

For *in vitro* diagnostic use only



Store in DARK at 2 to 8^oC

DO NOT FREEZE

K8003

Lyophilised Enzyme	5 vials
Enzyme Diluent	5 x 20mL
Colour Reagent	1 x 100mL
Aqueous Calibrator (standard) (2.0 mmol/L / 302 mg/L)	1 x 3.0mL
Bottle Adaptor	1 x

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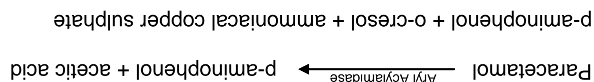
1. Clinical Application

Paracetamol (acetaminophen) 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 drug in the blood, between four and twelve hours after ingestion.[7-8]

2. Principle of the Assay

The method is based on the use of an enzyme specific for the amide bond of acylated 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.



Paracetamol —(Aryl Acylamidase)→ p-aminophenol + acetic acid

p-aminophenol + o-cresol + ammoniacal copper sulphate

→ indophenol (blue)

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.

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.

4. Use of the Kit

Reagent Preparation and Storage

Reconstitute each vial of lyophilised enzyme when required. Remove the cap of a vial of enzyme diluent and screw on the bottle adaptor. Remove the cap and stopper from a bottle of lyophilised enzyme and screw into the other end of the bottle adaptor. Invert two to three times to mix the contents and ensure that the enzyme pellet is dissolved. Allow to stand at room temperature for five minutes. Make sure that all of the reagent is in the diluent bottle when unscrewing the bottles from the bottle adaptor. This becomes Enzyme Reagent. After reconstitution the enzyme reagent is stable for 4 months at 2 - 8^oC or 1 month at 18 - 25^oC or until the expiry date, whichever is sooner.

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

Turbidity may develop in the working enzyme reagent on prolonged storage. This will not adversely affect the performance of the assay. Use the other reagents as supplied.

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.

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 each time a new kit is started or a new vial of paracetamol enzyme is reconstituted.

6. References

1. Ambre, J and Alexander, M; Liver toxicity after acetaminophen ingestion. J. Am. Med. Assoc. 238, 500-501 (1977).
2. Black, M; Acetaminophen hepatotoxicity. Gastroenterol. 78, 382-392 (1980).
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4. 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).
5. 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).
6. Meredith, T J Volans, G N; Paracetamol toxicity and its treatment. Recent Advances in Clinical Pharmacology 2, Edinburgh, Churchill Livingstone. 130-148 (1980).
7. Meredith, T J and Vale, J A; Paracetamol poisoning. Poisoning Diagnosis and Treatment, London, Update Books, 104-112 (1981).
8. Prescott, L F; Prevention of hepatic necrosis following paracetamol overdosage. Health Bulletin 4, 204-212 (1979).
9. Hammond, P M, Price, C P and Scawen, M D; Production of aryl acylamidase. US Patent Number US4430433 (1984).
10. 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).
11. 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).
12. 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)
13. Burtis, CA, Ashwood, ER editors; Tietz Textbook of Clinical Chemistry, 2nd Ed. Philadelphia, PA: WB Saunders; 1994:2212

Method

1. For each assay dispense 5µL (3-10µL) of either sample, calibrator or control into the respective cuvettes.
2. Add 100µL of Enzyme Reagent and incubate for 180 seconds.
3. Add 100µL of Colour Reagent and mix well then incubate for ten minutes. **Important:** The ratio of sample to Enzyme Reagent and Colour Reagent <u>must</u> be maintained otherwise interference from N-acetyl cysteine may occur.
4. Read the absorbance at 615nm (primary wavelength) and 700nm (secondary wavelength). A primary wavelength between 590nm - 640nm can be used depending on analyser availability.
5. The method uses a single standard calibration point. The values are expressed as mmol/L and the standard value is 2.0mmol/L (302mg/L). The assay is linear to 4.0mmol/L (604.8mg/L)

Calculation of Results

$$\text{Sample concentration (mmol/L)} = \frac{(\text{Sample absorbance} - B)}{(\text{Calibrator absorbance} - B)} \times S$$

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 generic method for running the assay on an automated platform. However, 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. A minimum of two levels of controls spanning the medical decision range is recommended to be run daily. If quality control results do not meet the acceptance criteria then recalibration may be necessary.

Therapeutic Range

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 patients[13].
Toxic concentrations are >1.99mmol/L (300mg/L) at four hours after ingestion and >0.33mmol/L (50mg/L) after 12 hours. For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Safety Precautions

For *in-vitro* diagnostic use only. For Professional use only.
Enzyme Diluent contains 0.1% 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 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:

	Conc (mmol/L)	S.D. (mmol/L)	Conc (mg/L)	S.D. (mg/L)	C.V. (%)
Intra assay	0.20	0.005	30.2	0.755	2.4
	0.37	0.004	55.9	0.604	1.1
	1.17	0.012	176.7	1.812	1.0
Inter assay	0.23	0.006	34.7	0.906	2.6
	0.41	0.006	61.9	0.906	1.5
	1.18	0.020	178.2	3.02	1.7

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

Accuracy

When patient serum samples were assayed and the results compared with those obtained by using the K8002 kit, the following regression equation resulted:

y = 0.999x - 1.39 (mmol/L), r = 0.9997, n = 35

where y = K8003 and x = K8002.

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 4.0 mmol/L (604.8mg/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.

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‡
acetylsalicylic acid†
amylobarbitone
amitryptyline
amphetamine
caffeine
chlordiazepoxide*
chlormezanone
chlorpropamide
dextropropoxyphene
diazepam*
dihydrocodeine
diphenydramine
impiramine
indomethacin
lorazepam*
meprobamate
methadone
methaqualone
nitrazepam*
oxypertine
p-aminosalicylic acid
pentazocine
p-ethoxyacetanilide(phenacetin)
p-ethoxyaniline (phenetidin)
phenobarbitone
phenytoin
promethazine
salicylamide
salicylic acid†
salicyluric acid
secobarbitone
sodium barbitone
theophylline
tolbutamide

* 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.