

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 | K8003 | |
| REAG | ENZ | Lyophilised Enzyme 5 vials |
| DIL | Enzyme Diluent 5 x 20mL | |
| REAG | COL | Colour Reagent 1 x 100mL |
| CAL | 1 | 0 mmol/L (0 mg/L) Sera Calibrator 2 x 0.5mL |
| CAL | 2 | 2 mmol/L (302 mg/L) Sera Calibrator 2 x 0.5mL |
| CAL | 2 mmol/L (302 mg/L) Aqueous Calibrator 1 x 3.0mL | |
| Bottle Adaptor | | 1 |



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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.

REAGENT PREPARATION AND STORAGE

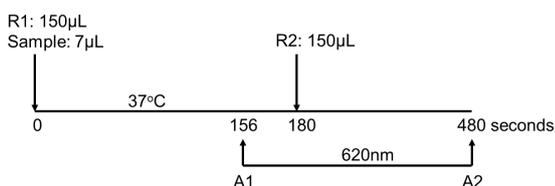
Reconstitute each vial of lyophilised enzyme when required. Remove the cap of a vial of enzyme diluent and screw on the bottle adaptor. Remove the cap and stopper from a bottle of lyophilised enzyme and screw into the other end of the bottle adaptor. Invert two to three times to mix the contents and 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 (R1)**. After reconstitution the enzyme reagent is stable for 4 months at 2 - 8°C or 1 month at 18 - 25°C or until the expiry date, whichever is sooner. **Colour Reagent (R2)** is ready to use.

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

ASSAY PROCEDURE

The paracetamol calibrators (standard) are included with the reagents and should be used each time a new kit is started or a new vial of paracetamol enzyme is reconstituted. Re-calibration is recommended every 7 days. Before each use, mix the reagents by gently inverting the vial. Avoid bubbles before placing on the clinical chemistry analysers. For clinical chemistry analyser protocols, the settings are; 2 point calibration with **CAL 1** set to **0mmol/L** (0mg/L) and **CAL 2** set to **2mmol/L** (302mg/L). Alternatively, the aqueous calibrator may be used with water or saline as the zero calibrator. Protocols are available for most clinical chemistry analysers. Please contact Customer Services at Cambridge Life Sciences or your local distributor for further information.

General Example



INTENDED USE

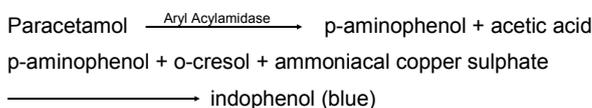
The Paracetamol (acetaminophen) assay is intended for the quantitative determination of paracetamol (acetaminophen) in human serum or plasma on clinical chemistry analysers or by manual spectrophotometric assays.

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

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.



SPECIMEN

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.

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 patients¹³. 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.

RESULTS

Paracetamol (acetaminophen) concentration is reported as mmol/L. To convert results to mg/L, use the following conversion factor: mmol/L x 0.151 = mg/L; mg/L ÷ 0.151 = mmol/L. The paracetamol (acetaminophen) value should be used in conjunction with information available clinical evaluations and other diagnostic procedures.

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.

PERFORMANCE

Data presented using CLS Paracetamol (acetaminophen) reagent were performed on an automated clinical chemistry analyser using an endpoint test mode.

Reportable Range

The reportable range is dependent on the sample to reagent ratio. The linearity is 0.02 - 5.00mmol/L (3 - 756mg/L). The regression equation against the target value is

$$y = 1.0292x - 3.41 \text{ (mg/L)}, r^2 = 0.9999$$

Limit of Detection

A drug-free sera sample was tested in 20 replicates and the mean + 3SD = 0.016mmol/L (2.45mg/L). LoD = 0.02mmol/L (3.0mg/L).

Recovery

Seven levels of paracetamol linearity material were run. The mean for each sample was determined and the % recovery calculated. Representative results are shown.

| Target (mmol/L) | Mean (mmol/L) | Difference (mmol/L) | Target (mg/L) | Mean (mg/L) | Difference (mg/L) | Recovery % |
|-----------------|---------------|---------------------|---------------|-------------|-------------------|------------|
| 0.31 | 0.313 | 0.003 | 46.87 | 47.32 | 0.44 | 100.9 |
| 0.62 | 0.632 | 0.012 | 93.74 | 95.49 | 1.75 | 101.9 |
| 1.26 | 1.252 | -0.008 | 190.51 | 189.03 | -1.48 | 99.2 |
| 2.52 | 2.522 | 0.002 | 381.02 | 380.89 | -0.13 | 100.0 |
| 3.00 | 3.052 | 0.052 | 453.60 | 458.81 | 5.21 | 101.6 |
| 5.00 | 5.152 | 0.028 | 756.00 | 779.00 | 23.00 | 103.0 |

Precision

Typical precision for the assay is as follows:

Three levels of commercial controls were assayed twice daily for 23 days. The measurements were used to calculate repeatability, between day, between run and total precision.

| Sample | Level 1 | Level 2 | Level 3 |
|---------------|---------|---------|---------|
| N | 46 | 46 | 46 |
| Mean (mmol/L) | 0.0857 | 0.2869 | 0.9003 |
| Mean (mg/L) | 12.94 | 43.32 | 135.95 |
| Repeatability | SD | 0.0023 | 0.0032 |
| | %CV | 2.68 | 1.12 |
| Between Day | SD | 0.0007 | 0.0039 |
| | %CV | 0.82 | 1.36 |
| Between Run | SD | 0.0027 | 0.0040 |
| | %CV | 3.15 | 1.39 |
| Total | SD | 0.0032 | 0.0060 |
| | %CV | 3.73 | 2.09 |

Accuracy

When patient serum samples were assayed and the results compared with those obtained by using the K8002 kit, the following regression equation resulted: where $y = K8003$ and $x = K8002$

$$y = 1.005x - 1.21 \text{ (mg/L)}, r^2 = 1.0000, n = 47$$

Correlation to an external quality scheme gave the following regression equation:

$$y = 1.025x - 4.45 \text{ (mg/L)}, r^2 = 0.9998, n = 23$$

Important: Although 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.

WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only.

For Professional use only.

Enzyme Diluent contains 0.1% o-cresol.

Signal Word: Danger
Hazard Statements:
H301 - Toxic if swallowed
H311 - Toxic in contact with skin
H314 - Causes severe skin burns and eye damage

Precautionary Statements:

P280 - Wear protective gloves/ eye protection/ face protection.

P301 + P310 - IF SWALLOWED: Immediately call a POISON CENTRE or doctor/physician.

P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 - Immediately call a POISON CENTRE or doctor/physician.

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.

The sera calibrators contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the sera calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used sera calibrators should be handled as clinical waste and incinerated or disposed of in accordance with local rules. It is recommended that gloves be worn when handling such items.

Safety data sheets are available upon request from your local representative.

Interferences

No interference was found using heparin, EDTA, fluoride oxalate and citrate blood collection tubes.

The common interfering endogenous substances of ascorbic acid, total bilirubin (unconjugated), direct bilirubin (conjugated), haemoglobin, triglycerides and intralipids show no significant interference up to the concentrations summarised in the table below.

Lipids may be removed using Lipoclear tubes taking into account the 20% dilution effect.

| Interferent | Concentration |
|--------------------------------|---------------|
| Ascorbic Acid | 1.76 g/L |
| Total Bilirubin (unconjugated) | 300.0 mg/L |
| Direct Bilirubin (conjugated) | 300.0 mg/L |
| Haemoglobin | 5.0 g/L |
| Triglycerides | 10.0 g/L |
| Intralipids | 1.0% (w/v) |

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* |
| amylobarbitone | oxypertine |
| amitriptyline | p-aminosalicylic acid |
| amphetamine | pentazocine |
| caffeine | p-ethoxyacetanilide (phenacetin) |
| chlormezanone | p-ethoxyaniline (phenetidine) |
| chlormezanone | phenobarbitone |
| chlorpropamide | phenytoin |
| dextropropoxyphene | promethazine |
| diazepam* | salicylamide |
| dihydrocodeine | salicylic acid† |
| diphenhydramine | salicylic acid |
| impiramine | secobarbitone |
| indomethacin | sodium barbitone |
| 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.

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.

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