PARACETAMOL (Acetaminophen) Assay Kit

Instructions for Use

For in vitro diagnostic use only

Store in DARK at 2 to 8°C

DO NOT FREEZE

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 and stopper from a bottle of lyophilised enzyme and add 10mL of enzyme diluent. Replace the stopper and swirl contents, inverting occasionally and ensure that the enzyme pellet is dissolved. Allow to stand at room temperature for five minutes. 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 A (R2) and Colour Reagent B (R3) are ready to use.

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

ASSAY PROCEDURE

The 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. 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: endpoint, 2 bubbles before placing on the clinical chemistry analysers.

Data presented using CLS Paracetamol (acetaminophen) reagent were preformed 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 - 3.00mmol/L (3 - 45mg/L). The regression equation against the target value is:

\[ y = 1.0014x + 0.32 \] (mg/L), \[ r^2 = 0.9999 \]

Limit of Detection

A drug-free sera sample was tested in 20 replicates and the mean ± 3SD = 0.012mmol/L (1.75mg/L). LoD = 0.02mmol/L (3.0mg/L).

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 commonly used analgesic which, if taken in excessive amounts, can lead to toxic liver damage and, less commonly, renal impairment. 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 mercapturic 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. The hepatocellular damage can be reduced by giving the patient compounds containing thiol groups such as methionine and N-acetyl cysteine. 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.

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-amino-phenol, which reacts specifically with o-cresol in ammoniacal copper solution to produce a blue colour.

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 anticogulants 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 patients. 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:

\[ \text{mg/L} = \frac{\text{mmol/L} \times 151}{\text{mmol/L} + 151} \]

Toxic concentrations are >1.99mmol/L (300mg/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

The reportable range is dependent on the sample to reagent ratio. The linearity is 0.02 - 3.00mmol/L (3 - 45mg/L). The regression equation against the target value is:

\[ y = 1.0014x + 0.32 \] (mg/L), \[ r^2 = 0.9999 \]

Limit of Detection

A drug-free sera sample was tested in 20 replicates and the mean ± 3SD = 0.012mmol/L (1.75mg/L). LoD = 0.02mmol/L (3.0mg/L).
Safety data sheets are available upon request from your local supplier and should be consulted before use. The product should be stored in accordance with local rules. If in contact with skin, wash with soap and water. 

**Precautionary Statements:**

H311: Toxic if swallowed

H314: Causing severe skin burns and eye damage

**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.

**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 from your local representative.**

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**Recovery (Linearity)**

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

<table>
<thead>
<tr>
<th>Target (mmol/L)</th>
<th>Mean (mmol/L)</th>
<th>Difference (mmol/L)</th>
<th>Target (mg/L)</th>
<th>Mean (mg/L)</th>
<th>Difference (mg/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.31</td>
<td>0.320</td>
<td>0.010</td>
<td>46.87</td>
<td>48.36</td>
<td>1.49</td>
<td>103.2</td>
</tr>
<tr>
<td>0.62</td>
<td>0.626</td>
<td>0.006</td>
<td>93.74</td>
<td>94.49</td>
<td>0.75</td>
<td>100.8</td>
</tr>
<tr>
<td>1.26</td>
<td>1.235</td>
<td>-0.025</td>
<td>190.51</td>
<td>186.51</td>
<td>-4.01</td>
<td>97.9</td>
</tr>
<tr>
<td>2.52</td>
<td>2.541</td>
<td>0.021</td>
<td>381.02</td>
<td>383.74</td>
<td>2.72</td>
<td>100.7</td>
</tr>
<tr>
<td>3.00</td>
<td>3.011</td>
<td>0.011</td>
<td>453.6</td>
<td>454.71</td>
<td>1.11</td>
<td>100.2</td>
</tr>
</tbody>
</table>

**Precision**

Typical precision for the assay is as follows:

Intra assay precision for twenty determinations and Inter assay precision was over 15 days.

<table>
<thead>
<tr>
<th>Conc (mmol/L)</th>
<th>Std Dev. (mmol/L)</th>
<th>Conc (mg/L)</th>
<th>Std Dev. (mg/L)</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>0.145</td>
<td>0.0050</td>
<td>16.73</td>
<td>0.748</td>
</tr>
<tr>
<td>Sample 2</td>
<td>0.260</td>
<td>0.0045</td>
<td>29.31</td>
<td>0.677</td>
</tr>
<tr>
<td>Sample 3</td>
<td>0.736</td>
<td>0.0066</td>
<td>111.18</td>
<td>0.992</td>
</tr>
<tr>
<td>Sample 4</td>
<td>0.054</td>
<td>0.0028</td>
<td>8.14</td>
<td>0.42</td>
</tr>
<tr>
<td>Sample 5</td>
<td>0.243</td>
<td>0.0044</td>
<td>28.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Sample 6</td>
<td>0.700</td>
<td>0.0069</td>
<td>105.78</td>
<td>1.04</td>
</tr>
</tbody>
</table>

**Accuracy**

When patient serum samples were assayed and the results compared with the K8003 kit, the following regression equation resulted: where y = K8002 and x = K8003 y = 0.9859 - 0.36 (mg/mL), r² = 0.9998, n = 47

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

y = 1.003x - 3.03 (mg/mL), r² = 0.9996, n = 23

**Interferences**

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

**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.

**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.

**WARNINGS AND PRECAUTIONS**

For in-vitro diagnostic use only. For Professional use only.

* Colour Reagent A contains 0.9% 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.

**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
- oxyperine
- amiltryptiline
- p-aminoacetic acid
- amphetamine
- penazocine
- caffeine
- p-ethoxyacetyl (phenacetin)
- chloridiazepoxide* p-ethylxylamine (phenetidin)
- chloromezamone
- phenobarbital
- chlorpropamide
- phenytin
- dextropropoxyphene
- promethazine
- diazepam*
- salicylamide
- dihydrocodeine
- salicylic acid†
- diphenidramine
- salicylic acid
- imipramine
- secobarbitone
- indomethacin
- sodium barbital
- lorazepam*
- theophylline
- mebrobarate
- 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.

**REFERENCES**