SALICYLATE
Enzyme Assay Kit

Instructions for Use
For in vitro diagnostic use only
Store in DARK at 2 to 8°C
DO NOT FREEZE

INTENDED USE
The Salicylate assay is intended for the quantitative determination of salicylate in human serum or plasma on clinical chemistry analysers or by manual spectrophotometric assays.

CLINICAL APPLICATION
Salicylate (aspirin) is a common non-steroidal drug used for its analgesic and anti-inflammatory properties. Ingestion of large amounts of salicylate leads to disturbances of the central nervous system and to gastrointestinal problems, encephalopathy and renal failure. Due to its accessibility, accidental or intentional ingestion by children and adults represents a major poisoning problem.1-4
Salicylate intoxication represents an acute medical emergency and rapid diagnosis and quantitation of the drug is necessary to assess effective patient management. Serum concentrations in excess of 4.4 mmol/L (607 mg/L) are usually lethal. Salicylate has traditionally been measured by the ‘Trinder’ reaction which is based on the interaction between salicylate and ferric ions.5-6 This test is not specific. This enzymatic Salicylate Assay provides a rapid, specific and simple method for salicylate determination.

PRINCIPLE OF THE ASSAY
Salicylate Hydroxylase catalyses the conversion of salicylate and NADH to catechol and NAD+ in the presence of oxygen. The resulting decrease in absorbance at 340nm, due to the conversion of NADH to NAD+, is directly proportional to the concentration of Salicylate in the sample.7

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.

REAGENT PREPARATION AND STORAGE
Reagents are supplied ready to use. The reagents should be clear; bubbles before placing on the clinical chemistry analysers. For clinical chemistry analyser protocols, the settings are: 2 point calibration with water or saline as the zero calibrator and CAL set to 1.5mmol/L (207mg/L) Aqueous Calibrator 1 x 2.0mL.

ASSAY PROCEDURE
The salicylate calibrator (standard) is included with the reagents and should be used each time a new kit is started or a new vial of reagents are used. 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 water or saline as the zero calibrator set to 0mmol/L (0mg/L) and CAL set to 1.5mmol/L (207mg/L).

The regents are stable until the expiry date stated when stored at 2 - 8°C.

RESULTS
Salicylate concentration is reported as mmol/L. To convert results to mg/L, use the following conversion factor: mmol/L x 138 = mg/L; mg/L ÷ 138 = mmol/L. The salicylate 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 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 Salicylate assay 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 0.05 - 10.0mmol/L (6.2 - 1380mg/L). The regression equation against the target value is

\[ y = 1.0025x - 2.69 \text{ (mg/L)} \]

Limit of Detection
A drug-free sera sample was tested in 20 replicates and the mean + 2SD = 0.045mmol/L (6.2mg/L). LoD = 0.045mmol/L (6.2mg/L).
Recovery

Seven levels of salicylate linearity material were run. The mean for each sample was determined and the % recovery calculated.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.288</td>
<td>0.288</td>
<td>0.288</td>
</tr>
<tr>
<td>Mean (mg/L)</td>
<td>39.70</td>
<td>156.22</td>
<td>448.24</td>
</tr>
</tbody>
</table>

Representative results are shown.

Precision

Typical precision for the assay is as follows:

- Three levels of commercial controls were assayed twice daily for 20 days. The measurements were used to calculate repeatability, between day, between run and total precision.

Accuracy

Correlation to an external quality scheme (NEQAS/WEQAS) gave the following regression equation: over the range 0 - 800 mg/L

\[ y = 1.038x - 4.223 \text{ (mg/L)}, \ r^2 = 0.998, n = 71 \]

where \( y = EQAS \) and \( x = \text{kit method} \).

The following compounds have not been tested:

- salicytchnenicloranide
- amylobarbitalone
- amphetamine
- chlorzepoxide
- chlorizemazone
- chlorpropamide
- dextropropoxynhe
- nitrazepam
- oxepertine
- pentazocine
- p-ethoxyacetanalidite
- phenobarbitalone
- dizepam
- dithydrocodeine
- irazepam
- meprobamate
- methadone
- methaqualone
- secobarbitalone
- sodium barbitone
- 2, 5 dihydroxyphenylacetyl

**WARNINGS AND PRECAUTIONS**

For in-vitro diagnostic use only.

For Professional use only.

No special precautions are needed with these reagents.

However, general care in reagent handling is recommended.

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.

**CALIBRATOR STANDARDISATION**

Salicylate calibrators are manufactured using primary calibration material, Salicylate (99.5% - 100.5%) that meets USP specifications. They are manufactured gravimetrically and tested against independent controls.

**REFERENCES**


**Interferences**

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.

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic Acid</td>
<td>1.76 g/L</td>
</tr>
<tr>
<td>Total Bilirubin (unconjugated)</td>
<td>300.0 mg/L</td>
</tr>
<tr>
<td>Direct Bilirubin (conjugated)</td>
<td>300.0 mg/L</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>5.0 g/L</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>10.0 g/L</td>
</tr>
</tbody>
</table>

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

The following substances when added at a concentration of 500mg/L to serum containing salicylate showed no interference:

- acetylsalicylic acid
- sodium EDTA
- ibuprofen
- α-ketobutyric acid
- phenol
- salicylamide
- sodium oxalate
- theophylline
- uric acid
- n-acetyl cysteine
- amitryptiline
- caffeine
- promethazine
- phenytoin
- diphenhydramine
- impiramine
- indomethacin
- tolbutamide
- phenetidine
- p-aminosalicylic acid and 2, 5 dihydroxybenzoic acid (gentisic acid) are measured by this assay at a concentration of 500mg/L.

Samples containing the following prescription drugs should not be used:

- Sulfapyridine
- Sulfasalazine
- Temozolomide
  - (an oral antineoplastic drug used to treat certain types of brain cancers)

Sample should be collected prior to sulfasalazine administration due to the potential for falsely elevated results and prior to sulfapyridine or temozolomide administration due to the potential for falsely depressed results.