

SALICYLATE

Enzyme Assay Kit



Instructions for Use

IVD

For *in vitro* diagnostic use only



Store in DARK at 2 to 8°C
DO NOT FREEZE

REF

K9001

REAG ENZ

Enzyme Reagent (R2)

2 x 40mL

REAG NADH

NADH

NADH Reagent (R1)

2 x 20mL

CAL

1.5mmol/L (207mg/L) Aqueous Calibrator 1 x 2.0mL



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

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.

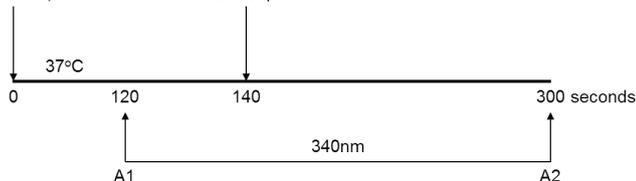
For clinical chemistry analyser protocols, the settings are; 2 point calibration with water or saline as the zero calibrator set to **0mmol/L** (0mg/L) and **CAL** set to **1.5mmol/L** (207mg/L).

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

R1: 75µL
H₂O: 165µL
Sample: 10µL

R2: 150µL



THERAPEUTIC RANGE

Normal therapeutic concentrations of aspirin and other salicylates depend upon what the drug is being used for, and blood levels must be interpreted in conjunction with the medical history and signs and symptoms. Lower blood levels are sufficient for pain relief and to lower risk of blood clots, but higher levels may be required for managing inflammation in rheumatic conditions such as arthritis. At these higher levels, some side-effects may become apparent. The severity of signs and symptoms, and at what dose they emerge, depends upon the individual. In general, the severity of salicylate toxicity increases with increasing concentrations. The table below summarizes some results that may be seen in a blood sample that is collected at least 4 hours after the last dose:

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 failure¹. Due to its accessibility, accidental or intentional ingestion by children and adults represents a major poisoning problem^{2,3,4}.

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 ions⁵. 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 sample⁶.



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; significant turbidity would indicate some deterioration in the reagent. The NADH Reagent is **R1** and the Enzyme Reagent is **R2**.

Salicylate result

0.145 - 0.725 mmol/L
(20 - 100 mg/L)
0.725 - 2.174 mmol/L
(100-300 mg/L)
> 2.174 mmol/L
(> 300 mg/L)

Result Interpretation

Therapeutic level for pain relief (analgesia)
Anti-inflammatory level; some symptoms of toxicity may appear, such as headache, tinnitus, vertigo
Symptoms of toxicity increasingly frequent

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

$$y = 1.0025x - 2.69 \text{ (mg/L)}, r^2 = 0.9995$$

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.

Target (mmol/L)	Mean (mmol/L)	Difference (mmol/L)	Target (mg/L)	Mean (mg/L)	Difference (mg/L)	Recovery %
0.034	0.108	0.074	4.72	14.97	10.25	
0.124	0.169	0.045	17.10	23.32	6.22	
0.303	0.328	0.025	41.87	45.26	3.39	108.1
0.662	0.626	-0.036	91.40	86.37	-5.03	94.5
1.380	1.325	-0.056	190.47	182.80	-7.67	96.0
2.816	2.697	-0.119	388.60	372.20	-16.40	95.8
5.687	5.550	-0.137	784.86	765.94	-18.92	97.6
11.430	11.536	0.105	1577.38	1591.91	14.52	100.9

Sample	Level 1	Level 2	Level 3
N	119	120	120
Mean (mmol/L)	0.288	1.132	3.248
Mean (mg/L)	39.70	156.22	448.24
Repeatability	SD	0.0136	0.0157
	%CV	4.7	1.4
Between Day	SD	0.0151	0.0311
	%CV	5.3	2.7
Between Run	SD	0.0052	0.0162
	%CV	1.8	1.4
Total	SD	0.0170	0.0341
	%CV	5.9	3.0

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 = kit method.

The following compounds have not been tested:

salicyphenolic glucuronide	amylobarbitone	amphetamine
chlordiazepoxide	chlormezanone	chlorpropamide
dextropropoxyphene	nitrazepam	oxypertine
pentazocine	p-ethoxyacetanilide	phenobarbitone
diazepam	dihydrocodeine	lorazepam
meprobamate	methadone	methaqualone
secobarbitone	sodium barbitone	2, 5 dihydroxyphenylacetate

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

1. Hammond, P M, Ramsay, J R, Price, C P, Campbell R S and Chubb S A P, A Simple Colorimetric Assay to Determine Salicylate Ingestion Utilising Salicylate Monooxygenase, Ann NY Acad Science (1987); 501; 288-291.
2. Done A K; Salicylate Intoxication. Pediatrics 26, 800 – 807 (1960).
3. Tietz NW, Fundamentals of Clinical Chemistry, pp1728,1856 (1976).
4. Nattelson S, Microtechniques of Clinical chemistry for the Routine Laboratory, pp332 (1957)
5. Trinder P, Biochem J. 57, pp301 (1947)
6. You, K, Bittikofer, J A, Quantitation of Salicylate in Serum by Use of Salicylate Hydroxylase, Clin Chem 30/9, 1549 – 1551 (1984).

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.

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

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:

acetaminophen	acetylsalicylic acid	sodium EDTA
ibuprofen	α -ketobutyric acid	methyl salicylate
phenol	salicylamide	sodium benzoate
sodium oxalate	theophylline	uric acid
n-acetyl cysteine	amitriptyline	caffeine
promethazine	phenytoin	diphenhydramine
imipramine	indomethacin	tolbutamide
phenitidine		

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 (occasionally used for dermatitis herpetiformis and related skin disorders)

Sulfasalazine (accepted treatment for inflammatory bowel disease, ulcerative colitis, Crohn's disease, rheumatoid arthritis, inflammatory arthritis and uveitis)

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.