

**Salicylate****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
20753580 322	Salicylate (150 tests)	System-ID 07 5358 0   Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 501/502
Materials required (but not provided):		
20759198 122	COBAS Salicylate Calibrators CAL A-B (2 x 3 mL)	Codes 638-639
04521536 190	TDM Control Set 1) Level I (2 x 5 mL) 2) Level II (2 x 5 mL) 3) Level III (2 x 5 mL)	Code 310 Code 311 Code 312

**English****System information**For **cobas c** 311/501 analyzers:**SALI** : ACN 780For **cobas c** 502 analyzer:**SALI** : ACN 8780**Intended use**In vitro test for the quantitative determination of toxic levels of salicylate in serum and plasma on Roche/Hitachi **cobas c** systems.**Summary**

Salicylate is a common drug used in many formulations due to its analgesic and anti-inflammatory properties. Salicylate overdose can cause metabolic acidosis with a high anionic gap, gastrointestinal and central nervous system disturbances, as well as encephalopathy and renal failure.<sup>1</sup> Therefore, a method for the rapid and accurate determination of salicylate is needed.

**Test principle**

This determination depends upon the conversion of salicylate in the presence of NADH by salicylate hydroxylase to catechol and NAD. The concomitant conversion of NADH to NAD is measured by the decrease in absorbance at 340 nm. The decrease is proportional to the concentration of salicylate present in the sample.

**Reagents - working solutions****R1** NADH 0.3 mmol/L, preservative**R2** Salicylate hydroxylase (microbial) ≥ 7000 U/L, preservative

R1 is in position B and R2 is in position C.

**Precautions and warnings**

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:

Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:

Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

**Reagent handling**

Ready for use

**Storage and stability**Shelf life at 2-8 °C: See expiration date on **cobas c** pack label

On-board in use and refrigerated on the analyzer: 26 weeks

**Do not freeze.****Specimen collection and preparation**

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum: Collect serum using standard sampling tubes.

Plasma: K<sub>2</sub>-EDTA and lithium heparin plasma.Stability:<sup>2</sup> at least 2 weeks at 4 °C

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not induce foaming of specimens. Specimens should not be repeatedly frozen and thawed.

Invert thawed specimens several times prior to testing.

**Materials provided**

See "Reagents – working solutions" section for reagents.

**Materials required (but not provided)**

See "Order information" section

General laboratory equipment

**Assay**

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

**Application for serum and plasma**

Deselect Automatic Rerun for these applications in the Utility menu, Application screen, Range tab.

**cobas c 311 test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 7-18		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Decrease		
Unit	µg/mL		
Reagent pipetting	Diluent (H <sub>2</sub> O)		
R1	100 µL	30 µL	
R2	5 µL	30 µL	
Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

**cobas c 501/502 test definition**

Assay type 2-Point End

**Salicylate**

Reaction time / Assay points	10 / 12-27	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Decrease	
Unit	µg/mL	
Reagent pipetting		Diluent (H <sub>2</sub> O)
R1	100 µL	20 µL
R2	5 µL	20 µL
<i>Sample volumes</i>	<i>Sample</i>	<i>Sample dilution</i>
		<i>Sample      Diluent (NaCl)</i>
Normal	2.5 µL	–      –
Decreased	2.5 µL	–      –
Increased	2.5 µL	–      –

**Calibration**

Calibrators	S1-2: COBAS Salicylate Calibrators
Calibration mode	Linear
Calibration frequency	2-point calibration - after <b>cobas c</b> pack change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against USP reference standards. The calibrators are prepared to contain known quantities of salicylate in buffer.

**Quality control**

For quality control, use control materials as listed in the "Order Information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

**Calculation**

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:<sup>3</sup> µg/mL x 0.00724 = mmol/L  
µg/mL x 0.1 = mg/dL

**Limitations - interference**

*Icterus, Hemolysis, Lipemia:*

The serum index cutoff values in the application settings are based on the salicylate concentration of 300 µg/mL (2.17 mmol/L) and should be adjusted to the intended use of the assay as appropriate.

Criterion: Recovery within ± 5 µg/mL (0.036 mmol/L) of initial value at a salicylate level of approximately **20 µg/mL** (0.145 mmol/L) for bilirubin and hemoglobin and at a salicylate level of approximately **40 µg/mL** (0.290 mmol/L) for lipemia.

Icterus:<sup>4</sup> No significant interference up to an I index of 23 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 393 µmol/L or 23 mg/dL).

Hemolysis:<sup>4</sup> No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Lipemia (Intralipid):<sup>4</sup> No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at a salicylate level of approximately **200 µg/mL** (1.45 mmol/L)

Icterus:<sup>4</sup> No significant interference up to an I index of 23 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 393 µmol/L or 23 mg/dL).

Hemolysis:<sup>4</sup> No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):<sup>4</sup> No significant interference up to an L index of 800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Criterion: Recovery within ± 10 % of initial value at a salicylate level of approximately **300 µg/mL** (2.17 mmol/L)

Icterus:<sup>4</sup> No significant interference up to an I index of 23 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 393 µmol/L or 23 mg/dL).

Hemolysis:<sup>4</sup> No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):<sup>4</sup> No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

**Other interferences:**

Criterion: Recovery within ± 10 % of initial value at a salicylate level of approximately 300 µg/mL (2.17 mmol/L)

Total protein: No interference from total protein up to 14 g/dL.

There is the possibility that other substances and/or factors may interfere with the test and cause unreliable results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>5</sup>

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**ACTION REQUIRED**

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCin1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

**Limits and ranges****Measuring range**

3.0-700 µg/mL (0.02-5.07 mmol/L)

Manually dilute samples above the measuring range 1 + 1 with the 0 µg/mL calibrator and reassay. Multiply the result by 2 to obtain the specimen value.

**Lower limits of measurement**

*Lower detection limit of the test*

3.0 µg/mL (0.02 mmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the 0 µg/mL calibrator (standard 1 + 2 SD, repeatability, n = 2).

**Expected values**

Serum concentrations exceeding 600 µg/mL (4.34 mmol/L) are usually lethal.<sup>6,7</sup> Salicylate intoxication occurs often in children due to its accessibility, in chronic patients with regular need of medication, or in patients who are taking combination prescription and nonprescription dosages. Overdosage of salicylate is also associated with suicide attempts in adolescents and adults.<sup>8</sup> Toxic manifestations have been observed at serum concentrations of > 270 µg/mL (> 1.95 mmol/L), and the toxic range is generally reported at > 300 µg/mL (> 2.17 mmol/L). The therapeutic range varies and has been reported to be 30 to 100 µg/mL (0.22 to 0.72 mmol/L) for anti-pyretic/analgesic conditions and 150 to 300 µg/mL (1.09 to 2.17 mmol/L) for anti-inflammatory/rheumatic fever conditions.<sup>9</sup>

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

**Specific performance data**

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

**Precision**

Precision was determined using human samples and controls in a modified NCCLS EP5-T2 protocol. Repeatability n = 21, intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained on a Roche/Hitachi **cobas c 501** analyzer.

*Serum/Plasma*

Repeatability	Mean µg/mL (mmol/L)	SD µg/mL (mmol/L)	CV %
Control 1	34.9 (0.253)	1.1 (0.008)	3.2
Control 2	152 (1.10)	2 (0.02)	1.5
Control 3	496 (3.59)	6 (0.04)	1.2
HS 1	46.2 (0.334)	2.3 (0.017)	5.0
HS 2	231 (1.67)	2 (0.02)	1.0

Intermediate precision	Mean µg/mL (mmol/L)	SD µg/mL (mmol/L)	CV %
Control 1	34.9 (0.253)	1.5 (0.011)	4.2
Control 2	152 (1.10)	3 (0.02)	2.1
Control 3	496 (3.59)	8 (0.06)	1.5
HS 1	46.2 (0.334)	2.8 (0.020)	6.1
HS 2	231 (1.67)	4 (0.029)	1.6

The data obtained on **cobas c 501** analyzer(s) are representative for **cobas c 311** analyzer(s).

**Method comparison****Serum/plasma**

Salicylate values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c 501** analyzer (y) were compared with those determined with the corresponding reagent on a COBAS INTEGRA 800 analyzer (x).

Sample size (n) = 70

Passing/Bablok<sup>10</sup>

y = 1.035x - 0.590 µg/mL

τ = 0.970

Linear regression

y = 1.067x - 3.31 µg/mL

r = 0.999

The sample concentrations were between 8.04 and 524 µg/mL (0.058 and 3.79 mmol/L).

The data obtained on **cobas c 501** analyzer(s) are representative for **cobas c 311** analyzer(s).

**Analytical specificity**

The following compounds were tested for cross-reactivity.

Compound	Concentration Tested (µg/mL)	% Cross- reactivity
Acetylsalicylic acid	1000	24.1
m-Aminosalicylate	1000	8.34
p-Aminosalicylate	1000	28.8
p-Anisic acid	1000	ND
Benzoic acid	1000	ND
Chlorzoxazone	500	0.65
Diflunisal	500	1.10
EDTA disodium	300	1.55
Gentisic acid	1000	2.86

Homogentisic acid	1000	1.89
alpha-Ketobutyric acid	1000	0.32
Methyl salicylate	1000	6.44
Naprosyn (Naproxen)	500	ND
Oxalic acid	300	1.22
Phenol	1000	0.33
Salicyluric acid	1000	1.96
Salicylamide	1000	0.49
Theophylline	300	1.43
Uric acid	300	ND

ND = not detected

Tests were performed on 15 drugs. No significant interference with the assay was found.

Acetaminophen	Ibuprofen
Acetyl cysteine	Levodopa
Ampicillin-Na	Methyldopa + 1.5 H <sub>2</sub> O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline
Doxycycline (Tetracycline)	

**References**

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- Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: <https://ec.europa.eu/tools/eudamed>

# SALI

## Salicylate



### Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see [dialog. Roche.com](http://dialog. Roche.com) for definition of symbols used):

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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