SALI

cobas®

Salicylate

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
20753580 322	Salicylate (150 tests)	System-ID 07 5358 0	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
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 780
For **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.[1] 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.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
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_2-EDTA and lithium heparin plasma.

Stability:[2]	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_2O)	
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
Reaction time / Assay points	10 / 12-27
Wavelength (sub/main)	700/340 nm
Reaction direction	Decrease
Unit	µg/mL

SALI

Salicylate

Reagent pipetting		Diluent (H_2O)	
R1	100 µL	20 µL	
R2	5 µL	20 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
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 **cobas c** 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:[3] 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:[4] 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:[4] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Lipemia (Intralipid):[4] 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:[4] 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:[4] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[4] 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:[4] 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:[4] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[4] 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.[5]

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-SmpCln1+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 = 21).

Expected values

Serum concentrations exceeding 600 µg/mL (4.34 mmol/L) are usually lethal.[6,7] 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.[8] 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.[9]

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

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[10]	Linear regression
y = 1.035x - 0.590 µg/mL	y = 1.067x - 3.31 µg/mL
τ = 0.970	r = 0.999

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

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_2O
Ascorbic acid	Metronidazole
Ca-Dobesilate	Phenylbutazone
Cefoxitin	Rifampicin
Cyclosporine	Theophylline
Doxycycline (Tetracycline)	

References

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3 Tietz NW. Clinical Guide to Laboratory Tests. 3rd ed. Philadelphia, PA: WB Saunders Co: 1995:856.

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10 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.

Symbols

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

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

SALI

Salicylate

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