SALI

cobas®

Salicylate

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058636190	08058636500	Salicylate (500 tests)	System-ID 2105 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

20759198122	COBAS Salicylate Calibrators CAL A-B (1 x 3 mL)	Codes 20638-20639	
04521536190	TDM Control Set Level I (2 x 5 mL) Level II (2 x 5 mL) Level III (2 x 5 mL)	 Code 20310 Code 20311 Code 20312	

English

System information
SALI: ACN 21050

Intended use
In vitro test for the quantitative determination of toxic levels of salicylate in serum and plasma on **cobas c** systems.

Summary
Salicylate measurements performed with this assay, in human serum and plasma, are used as an aid in the identification and management of salicylate intoxication.

Salicylate is a common analgesic, antipyretic and anti-inflammatory drug. Its prodrug, acetylsalicylic acid, is used in medicinal products and formulations. After ingestion, acetylsalicylic acid is hydrolyzed to salicylate, which is responsible for the therapeutic effects due to its ability to inhibit prostaglandin biosynthesis through irreversible inhibition of cyclooxygenase enzymes (COX-1 and COX-2 isoenzymes). Salicylate also reduces prostaglandin synthesis, therefore it has been recommended at low dosage as prophylactic therapy for individuals at risk for thromboembolic disease.[1]

The primary acid-base disturbance observed with salicylate overdose depends on age and severity of intoxication. Salicylate overdose can cause metabolic acidosis with an elevated anion gap, gastrointestinal and central nervous system disturbances, as well as encephalopathy and renal failure.[1,2]

Rapid and accurate determination of salicylate concentrations, together with the assessment of clinical features, is needed to guide the appropriate management of possible intoxication.[2,3,4,5]

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

R3 Salicylate hydroxylase (microbial) ≥ 7000 U/L, preservative

R1 is in position B and R3 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.

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:	12 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:[6] At least 2 weeks at 2-8 °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.

See the limitations and interferences section for details about possible sample interferences.

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

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	100 µL	20 µL
R3	5 µL	20 µL
Sample volumes	*Sample*	*Sample dilution*

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		Sample	*Diluent (NaCl)*
Normal	2.5 µL	–	–
Decreased	2.5 µL	–	–
Increased	2.5 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1-2: COBAS Salicylate Calibrators
Calibration mode	Linear
Calibration frequency	Full 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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks.

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

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors:[7] µg/mL x 0.00724 = mmol/L
µg/mL x 0.1 = mg/dL

Limitations - interference

Icterus, Hemolysis, Lipemia:

The sample index cutoff values in the application settings are based on a salicylate concentration of 20 µg/mL (0.145 mmol/L) for icterus and hemolysis and a salicylate level of 40 µg/mL (0.290 mmol/L) for lipemia.

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

Icterus:[8] 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:[8] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Lipemia (Intralipid):[8] 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 concentration of approximately **200 µg/mL** (1.45 mmol/L).

Icterus:[8] 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:[8] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8] 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 concentration of approximately **300 µg/mL** (2.17 mmol/L).

Icterus:[8] 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:[8] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[8] 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 concentration of approximately 300 µg/mL (2.17 mmol/L).

Total protein: No significant interference from total protein up to a concentration of 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.[9]

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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

5-700 µg/mL (0.04-5.07 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 4 µg/mL (0.03 mmol/L)
Limit of Detection	= 5 µg/mL (0.04 mmol/L)
Limit of Quantitation	= 10 µg/mL (0.07 mmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration salicylate samples.

Expected values

Serum concentrations exceeding 600 µg/mL (4.34 mmol/L) are usually lethal.[10,11] 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.[12] 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.[13]

Expected values reflect the data and information provided in the reference and do not necessarily represent therapeutic recommendations and/or dosage instructions. For therapeutic recommendations and dosage instructions refer to applicable guidelines and the full prescription information of the drug.

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Serum/plasma

Repeatability	*Mean µg/mL*	*SD µg/mL*	*CV %*
TDMC1[a)]	40.8	1.13	2.8
TDMC2[b)]	167	3.61	2.2
TDMC3[c)]	478	1.63	0.3
Human serum 1	6.75	0.918	13.6
Human serum 2	65.4	1.62	2.5
Human serum 3	234	1.82	0.8
Human serum 4	348	1.40	0.4
Human serum 5	598	2.81	0.5

Intermediate precision	*Mean µg/mL*	*SD µg/mL*	*CV %*
TDMC1[a)]	40.8	1.20	2.9
TDMC2[b)]	167	3.61	2.2
TDMC3[c)]	487	2.11	0.4
Human serum 1	6.48	0.959	14.8
Human serum 2	65.4	1.67	2.5
Human serum 3	234	1.87	0.8
Human serum 4	348	1.68	0.5
Human serum 5	598	3.29	0.6

a) TDM Control Set Level I

b) TDM Control Set Level II

c) TDM Control Set Level III

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Serum/plasma

Salicylate values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 69

Passing/Bablok[14]	Linear regression
y = 1.030x - 0.132 µg/mL	y = 1.020x + 0.398 µg/mL
τ = 0.971	r = 0.999

The sample concentrations were between 4.70 and 664 µg/mL.

Salicylate values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 67

Passing/Bablok[14]	Linear regression
y = 1.040x + 0.428 µg/mL	y = 1.057x - 2.62 µg/mL
τ = 0.971	r = 1.000

The sample concentrations were between 5.00 and 640 µg/mL.

Salicylate values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 68

Passing/Bablok[14]	Linear regression
y = 0.961x + 1.35 µg/mL	y = 0.940x + 4.48 µg/mL
τ = 0.987	r = 0.999

The sample concentrations were between 5.49 and 670 µg/mL.

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 detectable

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

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Doxycycline (Tetracycline)

References

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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

Symbols

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

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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