03246353001V2.0

Cl Electrode

Chloride

Order information

REF	CONTENT	Analyzer(s) on which the electrode can be used
03246353001	Cl Electrode 1 (electrode)	**cobas c** 311 analyzer **cobas** 6000 analyzer series: **cobas c** 501 module **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module **cobas pure** integrated solutions: **cobas c** 303 analytical unit **cobas pro** integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Materials required (but not provided):

03149501001	REF Electrode (1 electrode)	
11360981216	ISE Reference Electrolyte (5 x 300 mL) ①②	
10820652216	ISE Reference Electrolyte (1 x 500 mL) ③④	
08392013190	ISE Reference Electrolyte (2 x 2000 mL) ⑤⑥	
04522320190	ISE Internal Standard Gen.2 (5 x 600 mL) ①②	
04880455190	ISE Internal Standard Gen.2 (2 x 2000 mL) ③④⑤	
09137742190	ISE Internal Standard Gen.2 conc. (1 x 510 mL) ⑥	
05979854190	Internal Standard Insert - ISE (Set of 20) ①②	
04522630190	ISE Diluent Gen.2 (5 x 300 mL) ①②	
04880480190	ISE Diluent Gen.2 (2 x 2000 mL) ③④⑤	
11298500316	ISE Cleaning Solution (5 x 100 mL)	
20763071122	ISE Deproteinizer (6 x 21 mL) ④⑤⑥	
03110435180	Deproteinizer (1 x 125 mL) ⑥	
04663632190	Activator (9 x 12 mL)	
11183974216	ISE Standard Low (10 x 3 mL)	Code 20502
11183982216	ISE Standard High (10 x 3 mL)	Codes 20503, 20763
12149435122	Precinorm U Plus (10 x 3 mL)	Code 20300
12149443122	Precipath U Plus (10 x 3 mL)	Code 20301
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392

ISE reagents on:

① **cobas c** 311 analyzer

② **cobas** 6000 analyzer series: **cobas c** 501 module

③ **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

④ **cobas pure** integrated solutions: **cobas c** 303 analytical unit

⑤ **cobas pro** integrated solutions: **cobas pro** ISE analytical unit

⑥ **cobas pro** integrated solutions: **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

English

System information

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE CL	**ISE CL-U**	**ISE CL-P**	**ISE CL-S**
cobas c 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module	991	991	---	---

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE CL	**ISE CL-U**	**ISE CL-P**	**ISE CL-S**
cobas c 303 analytical unit, **cobas pro** ISE analytical unit	29090	29091	29092	29093

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	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	CL	**CL-U**	**CL-P**	**CL-S**
cobas ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit	29250	29251	29252	29253

Intended use

The Cl Electrode is a device intended for the in-vitro quantitative determination of chloride in human origin serum, plasma and urine.

Summary[1]

The chloride ion measurements may be useful for the diagnosis and monitoring purposes as an aid in diagnosing and monitoring chloride imbalance, including hypo- and hyperchloremia that can be associated with, or observed during a number of underlying disease states or pathological conditions.

Test principle

Ion-selective electrode, using automatically diluted serum/plasma or urine specimens. The chloride electrode is based on an ion exchanger.[2]

Calculation

The equation given below is used for the calculation of sample and/or QC results:

$$C_S = C_{IS} \times 10^{\frac{E_S - E_{IS}}{\pm S}}$$

Where:

C_S	concentration of the ion in the sample
C_{IS}	concentration of the ion in the ISE Internal Standard
E_S	EMF of the sample
E_{IS}	EMF of the ISE Internal Standard
S	Slope of the electrode

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration.

Precautions and warnings

For in vitro diagnostic use for trained laboratory technicians.

Warning

- Samples containing material of human origin are potentially infectious. Wear personal protective equipment when replacing or installing electrodes at analyzers. If any biohazardous material is spilled, wipe it up immediately and apply a disinfectant.
- If sample or waste contacts with your skin, wash the affected area immediately with soap and water, then apply a disinfectant. Consult a physician.
- When disposing of used electrodes, treat them as biohazardous.

Caution

- Do not use electrodes after the shelf life or on-board stability period has expired. Otherwise, it may lead to unstable sodium, potassium, and chloride results due to the unstable potential reading of electrodes.
- Perchlorate medication may result in falsely high chloride results due to an interference of perchlorate with the Cl Electrode determinations.
- Perform electrode flow path cleaning as stated in the Instructions for Use for applicable analyzers, at the end of a daily sample run. Improper electrode flow path cleaning may cause unstable reading of electrodes and it results in calibration failures.

As with any diagnostic test procedure, results should be interpreted taking all other test results and the clinical status of the patient into consideration.

In addition, pay attention to all precautions and warnings listed in the operator's manual of the analyzer.

NOTE: Boric acid (CAS Registry No. 10043-35-3) is contained in the gel solution inside the electrode at 0.2 % of the total weight as a preservative.

Storage and stability

Store at 7-40 °C.

See labels for expiration dates.

On-board stability

After installation the electrode is stable for the following time period:
2 months or 9000 tests, whichever comes first.

The electrodes should be replaced after this time period has expired. For replacement refer to instructions in the operator's manual of the applicable analyzers.

NOTE: When replacing the electrode in **cobas pro** or **cobas pure**, the user should scan the barcode affixed on the rear side of the package instead of the barcode placed on the product's label.

Slope range -40 to -68 mV/dec

NOTE: Due to the negative charge of the chloride ion, the slope is negative.

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.

It is important to follow tube manufacturers recommended procedures at and after blood collection.

Separate from cells if analysis is not performed within 4 hours.[3]

Serum

For chloride determinations, serum is the specimen of choice.

CAUTION: Serum separator tubes have to be used in accordance with the tube manufacturer's recommended procedures. If these procedures are not considered, it is possible to coat the sample probe with gel (interfering with proper sample level detection), or even to aspirate gel into the ISE system (resulting in a clogged system).

Plasma: Lithium heparin plasma

CAUTION: Inadequate mixing of plasma tubes can cause introduction of fibrin microclots into and subsequent clogging of the ISE.

NOTE: It is strongly recommended to avoid silicone-type gels, due to risk of silicon oil contaminations. In addition, tubes that exhibit a layer of clear liquid, which rises to the top of the serum after centrifugation, should not be used, in order to prevent coating the sample probes and interfering with ISE system. It is possible to clog the sample probes or the ISE tubing with gel or clots if these precautions are not taken.

Urine: Collect 24-hour urine without addition of preservatives and/or stabilizers. Store refrigerated during collection.

NOTE: Each laboratory should establish guidelines for determining acceptability of specimens and the corrective action to be taken if a specimen is considered unacceptable. Compile a laboratory-specific guideline.

Sample stability (serum, plasma): [4]

7 days at 15-25 °C

7 days at 2-8 °C

stable at (-15)-(-25) °C

Freeze/thaw only once.

Sample stability (urine): [4,5]

7 days at 15-25 °C

stable at (-15)-(-25) °C

up to 6 freeze-thaw cycles possible.[6]

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature[4] and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references

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and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See “Order information” section

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Application for serum, plasma and urine

Test definition

Serum/plasma

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 60-140 mmol/L

Analysis of chloride on ISE analytical units listed with serum and plasma specimens should yield a linear relationship from 60-140 mmol/L with a deviation from the linear line of less than 5 %.

The sample volumes given above under “Normal” are for samples, calibrators, and quality controls.

Urine

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
Decreased	6.5 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module		
Normal	10 µL	450 µL / ISE Diluent
Increased	15 µL	450 µL / ISE Diluent
cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
Decreased	10 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water
Decreased	10 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 20-250 mmol/L

Analysis of chloride on ISE analytical units listed with urine specimens should yield a linear relationship from 20-250 mmol/L with a deviation from the linear line of less than 10 %.

Determine samples having higher concentrations via the rerun function. Dilution of samples via rerun function is a 1:46 dilution. Results from samples diluted using the rerun function are automatically multiplied by the dilution factor.

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit for urine samples with decreased sample volume (Rerun):* 251-375 mmol/L.

Analysis of chloride on ISE analytical units listed with urine specimens should yield a linear relationship from 251-375 mmol/L with a deviation from the linear line of less than 10 %.

The sample volumes given above under “Normal” are for samples, calibrators, and quality controls.

Measuring range on *cobas 8000 ISE 900 / 1800 module:* 60-350 mmol/L

Analysis of chloride on **cobas** 8000 ISE 900 / 1800 module with urine specimens should yield a linear relationship from 60-350 mmol/L with a deviation from the linear line of less than 10 %.

Determine samples having lower concentrations via the rerun function. Dilution of samples via rerun function is a 1:31 dilution. Results from samples diluted using the rerun function are automatically multiplied by the dilution factor.

Measuring range on *cobas 8000 ISE 900 / 1800 module for urine samples with increased sample volume (Rerun):* 20-59.9 mmol/L

Analysis of chloride on **cobas** 8000 ISE 900 / 1800 module with urine specimens should yield a linear relationship from 20-59.9 mmol/L with a deviation from the linear line of less than 10 %.

The sample volumes given above under “Normal” are for samples and quality controls.

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

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 mmol/L
Limit of Detection	= 10 mmol/L
Limit of Quantitation	= 20 mmol/L

The Limit of Blank, the Limit of Detection and the 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 30 %. It has been determined using low concentration chloride samples.

Values below Limit of Quantitation are not reliable due to possible higher uncertainty.

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.

Calibration

Calibration requires the following calibrators: ISE Standard Low (S1), ISE Standard High (S2), and ISE Standard High (S3).

The slope of the calibration curve is calculated from Standards 1 and 2. ISE Internal Standard / ISE Internal Standard conc. is measured to provide E_{IS} for all measurements. Refer to the operator’s manual of the analyzer for detailed calibration instructions.

Traceability: ISE Standard Low and ISE Standard High are prepared gravimetrically from highly purified inorganic salts.

Purity of these salts has been certified by argentometric titration, acidimetric titration or perchloric acid titration.

Calibration frequency

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Calibration

- every 24 hours
- after ISE washing and maintenance
- after changing the reagent bottle ①
- after changing ISE Reference Electrolyte and/or Internal Standard conc. (depending on AutoCal settings) ②
- after replacing any electrode
- as required following quality control procedures

ISE reagents on:

① **cobas c** 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module, **cobas c** 303 analytical unit, **cobas pro** ISE analytical unit

② **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Refer to the operator's manual for a detailed description of the Calibration/AutoCal function.

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
	Precinorm U Plus, Precipath U Plus
Urine:	Quantitative urine controls are recommended for routine quality control.

Quality controls should be performed daily and after every additional calibration.

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.

Refer to appropriate value sheets/package inserts for additional information.

Traceability: Each Roche Diagnostics control listed above has been standardized against ISE Standard Low and ISE Standard High.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Hemolysis - serum/plasma

Hemolysis:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Hemolysis - urine

Hemolysis:[7] No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Icterus - serum/plasma

Icterus:[7] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Lipemia - serum/plasma

Lipemia (Intralipid, SMOFlipid):[7] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs

The following drugs have been tested and caused no significant interference when added to aliquots of pooled normal human serum up to the indicated concentration. Falsely high chloride values have been reported from patients receiving perchlorate medication. This is due to an interference of perchlorate ions with chloride ISE determinations.

Serum/plasma

Acetaminophen (Paracetamol)	200 mg/L
Acetylsalicylic acid	1000 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Cefoxitin	2500 mg/L
Cyclosporine	5 mg/L
Doxycyclin	50 mg/L
Heparin	5000 IU/L
Ibuprofen	500 mg/L
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
N-Acetylcysteine	1660 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Theophylline	100 mg/L

Urine

Acetaminophen (Paracetamol)	3000 mg/L
Ascorbic acid	4000 mg/L
Cefoxitin	12000 mg/L
Gentamycine sulfate	400 mg/L
Ibuprofen	4000 mg/L
Levodopa	1000 mg/L
Methyldopa	2000 mg/L
N-Acetylcysteine	10 mg/L
Ofloxacine	900 mg/L
Phenazopyridine	300 mg/L
Salicyluric acid	6000 mg/L
Tetracycline	300 mg/L

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.

Expected values[8]

Serum, Plasma	Adult	98-107 mmol/L
	>90 y	98-111 mmol/L
Urine 24 h	Infant	2-10 mmol/24 h
	Child <6 y	15-40 mmol/24 h
	6-10 y, M	36-110 mmol/24 h
	6-10 y, F	18-74 mmol/24 h
	10-14 y, M	64-176 mmol/24 h
	10-14 y, F	36-173 mmol/24 h
	Adult	110-250 mmol/24 h
	>60 y	95-195 mmol/24 h

The urinary excretion of chloride varies significantly with dietary intake. The values given here are typical of people on an average diet.

NOTE: It is recommended that each laboratory establishes and maintains its own reference ranges. The values given here are only to be used as a guideline.

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Precision

see precision data of the following analyzers in "Appendix 1: Precision":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Method comparison

see method comparison data of the following analyzers in "Appendix 2: Method comparison":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Maintenance

ISE washing procedure for cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 and cobas pro ISE analytical unit.

The system maintenance procedures and frequencies stated in the operator's manual of the respective analyzer must be performed each day at the end of the daily sample run or after an elevated sample throughput.

cobas c 311:	The specially designated positions on the sample disk are used.
Position W1:	ISE Cleaning Solution
Position W2:	Activator

The ISE Wash procedure has to be manually selected out of maintenance items.

cobas c 501:	The specially labeled wash rack (green) is used.
Position 1:	Multiclean (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas 8000 ISE:	The specially labeled wash rack (green) is used.
Position 1:	Cell Cleaning Solution (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas c 303, cobas pro ISE:	The specially labeled wash rack (green) is used.
Position 1:	ISE Cleaning Solution (used for weekly wash rack)
Position 2:	ISE Cleaning Solution (used for daily wash rack)
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

The ISE systems require conditioning after cleaning and prior to calibration.

NOTE: Always use fresh solutions for cleaning.

ISE washing procedure for cobas ISE neo analytical unit

cobas ISE neo:	The ISE system wash tube holder is used.
Position CS:	ISE Cleaning Solution
Position A:	Activator

The maintenance task "ISE system wash" is scheduled and initiated automatically. For detailed description, refer to the operator's manual.

On-board stability of auxiliary reagents: ISE Cleaning Solution 4 days, Activator 4 days.

NOTE: Always exchange the tubes on the ISE tube holder, using new tubes for fresh reagents. **You must not refill them**, as this will lead to deterioration of the ISE measuring unit(s). Refer to the operator's manual for further information.

Appendix 1: Precision

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

	Repeatability			Intermediate precision		
Sample (on a **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	68.5	0.2	0.3	68.5	0.6	0.8
Plasma medium	129.0	0.4	0.3	129.0	0.6	0.5
Plasma high	139.0	0.3	0.2	139.0	0.6	0.4
Precinorm U	86.2	0.2	0.3	86.2	0.5	0.6
Precipath U	119.2	0.3	0.2	119.2	0.5	0.4
Urine low	25.8	0.1	0.2	25.8	0.6	2.3
Urine medium	131.4	0.3	0.2	131.4	0.7	0.5
Urine high	243.4	0.6	0.2	243.4	1.8	0.7
Liquichek 1	97.5	0.2	0.2	97.5	1.6	1.6
Liquichek 2	198.2	0.4	0.2	198.2	2.3	1.2

cobas 8000 modular analyzer series: cobas 8000 ISE 900 / 1800 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

	Repeatability			Intermediate precision		
Sample (on a **cobas** 8000)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	67.1	0.3	0.4	67.1	0.6	1.0
Plasma medium	128.4	0.4	0.3	128.4	0.7	0.6
Plasma high	138.0	0.6	0.4	138.0	0.9	0.7
Precinorm U	77.1	0.3	0.4	77.1	0.6	0.8

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	Repeatability			Intermediate precision		
Sample (on a **cobas** 8000)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Precipath U	111.8	0.3	0.3	111.8	0.6	0.6
Urine low[1)]	21.6	0.2	1.0	21.6	0.8	3.7
Urine medium[2)]	167.6	0.5	0.3	167.6	1.1	0.7
Urine high[2)]	333.5	1.6	0.5	333.5	3.5	1.0
Liquichek 1[2)]	97.5	0.5	0.5	97.5	0.9	0.9
Liquichek 2[2)]	193.2	1.5	0.8	193.2	2.0	1.0

1) Data obtained with urine rerun function.

2) Data obtained with default urine mode.

cobas pure integrated solutions: cobas c 303 analytical unit

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

cobas pro integrated solutions: cobas pro ISE analytical unit

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 pro** ISE analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas pro** ISE analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	82.5	0.31	0.4	82.5	1.22	1.5
PCCC2[b)]	112	0.46	0.4	112	1.15	1.0
Human plasma 1	71.2	0.31	0.4	71.2	1.20	1.7
Human plasma 2	112	0.51	0.5	112	1.06	0.9
Human plasma 3	91.6	0.44	0.5	91.6	1.38	1.5
Human plasma 4	123	0.50	0.4	123	0.85	0.7
Human plasma 5	137	0.53	0.4	137	1.03	0.7
Human serum 1	73.4	0.23	0.3	73.4	1.08	1.5
Human serum 2	111	0.53	0.5	111	0.98	0.9
Human serum 3	91.4	0.35	0.4	91.4	1.17	1.3
Human serum 4	124	0.54	0.4	124	0.89	0.7
Human serum 5	133	0.62	0.5	133	0.82	0.6
Liquichek 1	95.2	0.41	0.4	95.2	1.18	1.2
Liquichek 2	184	0.69	0.4	184	1.79	1.0
Human urine 1	28.5	0.14	0.5	28.5	1.08	3.8
Human urine 2	139	0.58	0.4	139	1.44	1.0
Human urine 3	115	0.55	0.5	115	1.37	1.2
Human urine 4	207	0.93	0.5	207	1.92	0.9
Human urine 5	236	0.99	0.4	236	2.59	1.1

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

cobas pro integrated solutions: cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit

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 ISE neo** analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas** ISE neo analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	85.9	0.56	0.7	85.9	1.21	1.4
PCCC2[b)]	107	0.65	0.6	107	1.27	1.2
Human serum 1	68.0	0.32	0.5	68.0	1.07	1.6
Human serum 2	104	0.46	0.4	104	0.94	0.9
Human serum 3	97.8	0.46	0.5	97.8	0.94	1.0
Human serum 4	114	0.61	0.5	114	1.05	0.9
Human serum 5	138	0.65	0.5	138	1.11	0.8
Human plasma 1	65.0	0.30	0.5	65.2	1.20	1.8
Human plasma 2	101	0.63	0.6	101	1.07	1.1
Human plasma 3	94.5	0.54	0.6	94.4	1.02	1.1
Human plasma 4	110	0.50	0.5	110	1.14	1.0
Human plasma 5	135	0.74	0.5	135	1.28	0.9
Liquichek 1	82.3	0.40	0.5	82.0	0.99	1.2
Liquichek 2	193	1.14	0.6	193	2.12	1.1
Human urine 1	23.5	0.54	2.3	23.5	1.63	7.0
Human urine 2	132	0.57	0.4	132	1.33	1.0
Human urine 3	105	0.66	0.6	106	1.10	1.0
Human urine 4	201	0.93	0.5	202	2.01	1.0
Human urine 5	247	1.02	0.4	247	3.51	1.4

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Appendix 2: Method comparison

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

ISE values for human plasma and urine samples obtained on **cobas c** 501 analyzers (y) using ISE Standard High (compensated) as S3 Calibrator, were compared to those determined with the corresponding reference method (x) and with a **cobas c** 501 analyzer using ISE Compensator as S3 Calibrator.

The reference method used was: Chloride Analyzer 926S for chloride.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[9]	Coeff. (r)
x: coulometry y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 105	62.0	136	y = 1.033x -1.800	0.998
Bias at 90 mmol/L = 1.170 (1.3 %) Bias at 112 mmol/L = 1.896 (1.7 %)					
x: **cobas c** 501 (S3 = ISE Compensator) y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 105	61.4	138	y = 1.000x + 0.500	0.999

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Chloride

Bias at 90 mmol/L = 0.500 (0.6 %) Bias at 112 mmol/L = 0.500 (0.4 %)					
x: coulometry y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 105	22.0	248	y = 1.020x - 1.700	0.999
Bias at 60 mmol/L = -0.500 (-0.8 %) Bias at 170 mmol/L = 1.700 (1.0 %)					
x: **cobas c** 501 (S3 = ISE Compensator) y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 105	21.2	250	y = 0.989x + 0.669	1.000
Bias at 60 mmol/L = 0.009 (0.0 %) Bias at 170 mmol/L = -1.201 (-0.7 %)					

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas 8000 modular analyzer series: cobas 8000 ISE 900 / 1800 module

ISE values for human plasma and urine samples obtained on a **cobas** 8000 analyzer (y) using ISE Standard High as S3 Calibrator, were compared with those determined using the corresponding reference method (x) and with **cobas c** 501 (x) using ISE Standard High as S3 Calibrator.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[9]	Coeff. (r)
x: coulometry y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	65.0	123.0	y = 1.075x - 6.025	0.9902
Bias at 90 mmol/L = 0.725 (0.8 %) Bias at 112 mmol/L = 2.375 (2.1 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	61.9	127.9	y = 0.987x + 1.858	0.9984
Bias at 90 mmol/L = 0.688 (0.8 %) Bias at 112 mmol/L = 0.402 (0.4 %)					
x: coulometry y: **cobas** 8000 (S3 = ISE Standard High)	Urine[2)] / 108	66.0	313.0	y = 1.036x - 4.891	0.9995
Bias at 60 mmol/L = -2.731 (-4.6 %) Bias at 170 mmol/L = 1.229 (0.7 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Urine[2)] / 108	62.0	349.8	y = 0.908x + 9.018	0.9999
Bias at 60 mmol/L = 3.497 (5.8 %) Bias at 170 mmol/L = -6.623 (-3.9 %)					
x: coulometry y: **cobas** 8000 (S3 = ISE Standard High)	Urine[1)] / 92	22.0	59.0	y = 0.973x - 0.927	0.9987
Bias at 30 mmol/L = -1.737 (-5.8 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Urine[1)] / 92	20.2	57.3	y = 0.981x + 0.728	0.9992
Bias at 30 mmol/L = 0.158 (0.5 %)					

1) Data obtained with urine rerun function.

2) Data obtained with default urine mode.

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pure integrated solutions: cobas c 303 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas c** 303 ISE unit (y) were compared with those determined using **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas c** 303 ISE unit (y) were compared with a **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[9]	Coeff. (r)
x: **cobas pro** ISE y: **cobas c** 303 ISE	Plasma / 120	65.5	137	y = 0.996x + 0.276	0.999
Bias at 95 mmol/L = -0.110 (-0.1 %) Bias at 110 mmol/L = -0.171 (-0.2 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Plasma / 120	66.4	138	y = 1.000x - 1.20	0.999
Bias at 95 mmol/L = -1.20 (-1.3 %) Bias at 110 mmol/L = -1.20 (-1.1 %)					
x: **cobas pro** ISE y: **cobas c** 303 ISE	Serum / 118	62.2	138	y = 1.000x - 0.200	1.000
Bias at 95 mmol/L = -0.200 (-0.2 %) Bias at 110 mmol/L = -0.200 (-0.2 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Serum / 118	62.6	138	y = 1.003x - 1.01	1.000
Bias at 95 mmol/L = -0.761(-0.8 %) Bias at 110 mmol/L = -0.722 (-0.7 %)					

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Chloride

x: **cobas pro** ISE y: **cobas c** 303 ISE	Urine / 119	21.3	243	y = 1.011x - 1.03	1.000
x: **cobas c** 501 y: **cobas c** 303 ISE	Urine / 118	21.3	249	y = 0.984x + 2.29	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas pro ISE analytical unit

ISE values for human plasma samples obtained on a **cobas pro** ISE analytical unit (y) were compared with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas pro** ISE analytical unit (y) were compared with a **cobas c** 501 analyzer (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[9]	Coeff. (r)
x: **cobas c** 501 y: **cobas pro** ISE	Plasma / 118	60.5	140	y = 0.997x - 0.127	1.000
Bias at 95 mmol/L = -0.384 (-0.4 %) Bias at 110 mmol/L = -0.423 (-0.4 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Serum / 118	61.7	135	y = 1.000x - 0.600	1.000
Bias at 95 mmol/L = -0.600 (-0.6 %) Bias at 110 mmol/L = -0.600 (-0.5 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Urine / 119	25.0	245	y = 1.023x - 2.09	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas** ISE neo analytical unit (y) were compared with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

ISE values for human urine samples obtained on a **cobas** ISE neo analytical unit (y) were compared with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[9]	Coeff. (r)
x: **cobas c** 501 y: **cobas** ISE neo	Serum / 115	63.1	139	y = 1.021x - 1.41	0.998
Bias at 95 mmol/L = 0.593 (0.6 %) Bias at 110 mmol/L = 0.908 (0.8 %)					
x: **cobas pro** ISE y: **cobas** ISE neo	Serum / 115	63.1	136	y = 1.045x - 2.77	0.998
Bias at 95 mmol/L = 1.52 (1.6 %) Bias at 110 mmol/L = 2.19 (2.0 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Plasma / 119	62.4	137	y = 1.010x + 0.152	0.999
Bias at 95 mmol/L = 1.14 (1.2 %) Bias at 110 mmol/L = 1.29 (1.2 %)					
x: **cobas pro** ISE y: **cobas** ISE neo	Plasma / 118	60.7	135	y = 1.012x + 2.10	0.999
Bias at 95 mmol/L = 3.21 (3.4 %) Bias at 110 mmol/L = 3.38 (3.1 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Urine / 118	22.6	248	y = 1.004x - 1.20	1.000
x: **cobas pro** ISE y: **cobas** ISE neo	Urine /114	30.0	248	y = 1.008x - 0.843	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

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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

Cl Electrode

Chloride

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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):

Cont.	Quantity contained in the package
CONTENT	Quantity contained in the package
GTIN	Global Trade Item Number
INSTALL BEFORE	Latest date by which the electrode has to be installed on the analyzer
RoHS	Directive for the restriction of the use of certain hazardous substances in electrical and electronic equipment

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

CE 0123

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Roche

CREJ2

Creatinine Jaffé Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057532190*	08057532500	Creatinine Jaffé Gen.2 (2500 tests)	System-ID 2047 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057532214*	08057532500	Creatinine Jaffé Gen.2 (2500 tests)	System-ID 2047 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
03121313122	Precinorm PUC (4 x 3 mL)	Code 20240	
03121291122	Precipath PUC (4 x 3 mL)	Code 20241	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

CREJ2: ACN 20470 (Serum/plasma)

CREJ2U: ACN 20471 (Urine)

Intended use

In vitro test for the quantitative determination of creatinine in human serum, plasma and urine on **cobas c** systems.

Summary

Creatinine measurements, performed with this assay, in human serum, plasma and urine are used as an aid in diagnosis and monitoring of renal disease and in monitoring of renal dialysis. Creatinine measurements are also used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase).

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not reabsorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted. Its concentration is thus, inversely related to glomerular filtration rate (GFR).[1,2]

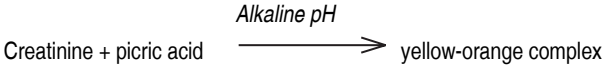
The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or decreased glomerular filtration rate (GFR) (less than 60 mL/min per 1.73 m^2) for 3 months or more.[2,3]

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR (eGFR) based only on the creatinine concentration in serum or plasma have been made.[4] Among the various approaches suggested, three have found wide recognition: the Cockroft and Gault, the Modification of Diet in Renal Disease (MDRD) Study equation and the CKD-EPI (Chronic Kidney Disease Epidemiology) equation. While the Cockcroft and Gault equation was derived from data in which serum creatinine was measured with the conventional Jaffé method, the MDRD study equation measured serum creatinine using the Jaffé method calibrated to an isotope dilution mass spectrometry (IDMS).[5,6] These estimates of GFR are useful during monitoring of renal dialysis.[7,8] In children, the Bedside Schwartz formula should be used.[9,10,11]

In addition to the diagnosis and treatment of renal disease and the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.[2]

Test principle[12,13,14]

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses "rate-blanking" to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -26 µmol/L (-0.3 mg/dL).



Reagents - working solutions

R1 Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5; preservative; stabilizer

R3 Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H314 Causes severe skin burns and eye damage.

Prevention:

P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P301 + P330 + P331	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.
P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 weeks

Specimen collection and preparation[15]

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

Only the specimens listed below were tested and found acceptable.

Serum.

Plasma: Li-heparin and K_2-EDTA plasma.

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.

Urine.

Collect urine without using additives. If urine must be collected with a preservative for other analytes, only hydrochloric acid (14 to 47 mmol/L urine, e.g. 5 mL 10 % HCl or 5 mL 30 % HCl per liter urine) or boric acid (81 mmol/L, e.g. 5 g per liter urine) may be used. If stabilizers are added to the sample, the sample index feature must not be used.

Stability in *serum/plasma:*[16]	7 days at 15-25 °C
	7 days at 2-8 °C
	3 months at -20 °C (± 5 °C)

Freeze only once.

Stability in *urine* (without preservative):[16]	2 days at 15-25 °C
	6 days at 2-8 °C
	6 months at -20 °C (± 5 °C)

Freeze only once.

Stability in *urine* (with preservative):	3 days at 15-25 °C
	8 days at 2-8 °C
	3 weeks at -20 °C (± 5 °C)

Freeze only once.

Centrifuge samples containing precipitates before performing the assay.

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

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)	570/505 nm	
Reagent pipetting		Diluent (H_2O)
R1	10 µL	58 µL
R3	13 µL	23 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	7.5 µL	–	–
Decreased	7.5 µL	20 µL	80 µL
Increased	7.5 µL	–	–

Application for urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	570/505 nm	
Reagent pipetting		Diluent (H_2O)
R1	10 µL	58 µL
R3	13 µL	23 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	7.5 µL	4 µL	96 µL
Decreased	7.5 µL	1.5 µL	135 µL
Increased	7.5 µL	4 µL	96 µL

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

Calibration

Application for serum/plasma (ACN 20470)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear

Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - every 8 weeks on-board - as required following quality control procedures

Application for urine (ACN 20471)
Transfer of calibration from serum/plasma application (ACN 20470)

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

Traceability: This method has been standardized against ID/MS.

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Precinorm PUC, Precipath PUC

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 26 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 in the unit µmol/L (mg/dL, mmol/L, mg/L).

Conversion factors:	µmol/L x 0.0113 = mg/dL
	µmol/L x 0.001 = mmol/L
	µmol/L x 0.113 = mg/L

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a creatinine concentration of 80 µmol/L (0.90 mg/dL) in serum/plasma and 2.5 mmol/L (28.3 mg/dL) in urine.

Serum/plasma

Icterus (*CREJ2*):[17] No significant interference up to an I index of 5 for conjugated bilirubin and 10 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 86 µmol/L or 5 mg/dL; approximate unconjugated bilirubin concentration: 171 µmol/L or 10 mg/dL).

Hemolysis:[17] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[17] No significant interference up to an L index of 800. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Pyruvate: No significant interference from pyruvate up to a concentration of 0.3 mmol/L (2.6 mg/dL).

Glucose: No significant interference from glucose up to a concentration of 25 mmol/L (450 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 5 mmol/L (88 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]

Exception: Antibiotics containing cephalosporin lead to significant false-positive values.[20,21] Cefoxitin causes artificially high creatinine results. Cyanokit (Hydroxocobalamin) may cause interference with results.

Values < 15 µmol/L (< 0.17 mg/dL) or negative results are reported in rare cases in children < 3 years and in elderly patients. In such cases use the Creatinine plus test to assay the sample.

Do not use Creatinine Jaffé for the testing of creatinine in hemolyzed samples from neonates, infants or adults with HbF levels ≥ 60 mg/dL for *CREJ2* applications.[22] In such cases, use the Creatinine plus test (≤ 600 mg/dL HbF) to assay the sample.

Estimation of the Glomerular Filtration Rate (GFR) on the basis of the Schwartz Formula can lead to an overestimation.[23]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[24]

The presence of ketone bodies can cause artificially high results in serum and plasma.

Urine

Icterus: No significant interference up to a conjugated bilirubin concentration of 855 µmol/L or 50 mg/dL.

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

Glucose: No significant interference from glucose up to a concentration of 120 mmol/L (2162 mg/dL).

Urea: No significant interference from urea up to a concentration of 2100 mmol/L (12612 mg/dL).

Urobilinogen: No significant interference from urobilinogen up to a concentration of 676 µmol/L (40 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19]

Exception: Cyanokit (Hydroxocobalamin) may cause interference with results.

High homogentisic acid concentrations in urine samples lead to false results.

The presence of ketone bodies can cause artificially high results in urine.

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

Serum/plasma

15-2200 µmol/L (0.17-24.9 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Urine

0.375-55 mmol/L (4.2-622 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3.6 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.6.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma (CREJ2)

Limit of Blank	= 15 µmol/L (0.17 mg/dL)
Limit of Detection	= 15 µmol/L (0.17 mg/dL)
Limit of Quantitation	= 15 µmol/L (0.17 mg/dL)

Urine (CREJ2U)

Limit of Blank	= 0.375 mmol/L (4.24 mg/dL)
Limit of Detection	= 0.375 mmol/L (4.24 mg/dL)
Limit of Quantitation	= 0.375 mmol/L (4.24 mg/dL)

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 creatinine samples.

Expected values

µmol/L

Serum/plasma

Adults[25]

Females	44-80 µmol/L
Males	62-106 µmol/L

Children[26]

Neonates (premature)	25-91 µmol/L
Neonates (full term)	21-75 µmol/L
2-12 months	15-37 µmol/L
1- < 3 years	21-36 µmol/L
3- < 5 years	27-42 µmol/L
5- < 7 years	28-52 µmol/L
7- < 9 years	35-53 µmol/L
9- < 11 years	34-65 µmol/L
11- < 13 years	46-70 µmol/L
13- < 15 years	50-77 µmol/L

mmol/L

Urine

1st morning urine[25]

Females	2.47-19.2 mmol/L
Males	3.45-22.9 mmol/L

24-hour urine[27]

Females	7.0-14.0 mmol/24 h
Males	9.0-21.0 mmol/24 h

Creatinine clearance[27,28]	71-151 mL/min

Refer to reference for a prospective study on creatinine clearance in children.[29]

mg/dL

Serum/plasma

Adults[25]

Females	0.50-0.90 mg/dL
Males	0.70-1.20 mg/dL

Children[26]

Neonates (premature)	0.29-1.04 mg/dL
Neonates (full term)	0.24-0.85 mg/dL
2-12 months	0.17-0.42 mg/dL
1- < 3 years	0.24-0.41 mg/dL
3- < 5 years	0.31-0.47 mg/dL
5- < 7 years	0.32-0.59 mg/dL
7- < 9 years	0.40-0.60 mg/dL
9- < 11 years	0.39-0.73 mg/dL
11- < 13 years	0.53-0.79 mg/dL
13- < 15 years	0.57-0.87 mg/dL

Urine

1st morning urine[25]

Females	28-217 mg/dL
Males	39-259 mg/dL

24-hour urine[27]

Females	740-1570 mg/24 h
Males	1040-2350 mg/24 h

Creatinine clearance[27,28]	71-151 mL/min

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 (CREJ2)

Repeatability	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[a)]	90.4	1.57	1.7
PCCC2[b)]	347	3.87	1.1
Human serum 1	48.2	1.40	2.9
Human serum 2	71.8	1.51	2.1
Human serum 3	480	4.15	0.9
Human serum 4	1064	12.0	1.1
Human serum 5	1791	20.6	1.2

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[a)]	89.2	2.33	2.6
PCCC2[b)]	347	5.24	1.5
Human serum 1	48.2	1.64	3.4
Human serum 2	71.8	1.89	2.6
Human serum 3	480	7.59	1.6
Human serum 4	1064	16.1	1.5
Human serum 5	1791	30.0	1.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

CREJ2

Creatinine Jaffé Gen.2

Urine (CREJ2U)

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PN PUC[c)]	8.89	0.0922	1.0
PP PUC[d)]	4.56	0.0560	1.2
Human urine 1	1.19	0.0310	2.6
Human urine 2	2.41	0.0311	1.3
Human urine 3	22.5	0.210	0.9
Human urine 4	28.4	0.318	1.1
Human urine 5	49.7	0.480	1.0

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PN PUC[c)]	8.89	0.151	1.7
PP PUC[d)]	4.56	0.0824	1.8
Human urine 1	1.19	0.0341	2.9
Human urine 2	2.43	0.0398	1.6
Human urine 3	22.5	0.339	1.5
Human urine 4	28.4	0.417	1.5
Human urine 5	49.7	0.745	1.5

c) Precinorm PUC

d) Precipath PUC

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

Creatinine values for human serum, plasma and urine 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).

Serum/plasma (CREJ2)

Sample size (n) = 71

Passing/Bablok[30]	Linear regression
y = 1.012x - 3.68 µmol/L	y = 1.010x - 3.19 µmol/L
τ = 0.980	r = 1.000

The sample concentrations were between 23.2 and 2133 µmol/L.

Urine (CREJ2U)

Sample size (n) = 72

Passing/Bablok[30]	Linear regression
y = 1.065x - 0.0368 mmol/L	y = 1.056x + 0.00514 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.388 and 50.8 mmol/L.

Creatinine values for human serum, plasma and urine 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).

Serum/plasma (CREJ2)

Sample size (n) = 70

Passing/Bablok[30]	Linear regression
y = 1.018x - 5.48 µmol/L	y = 1.015x - 4.42 µmol/L
τ = 0.968	r = 1.000

The sample concentrations were between 24.1 and 2114 µmol/L.

Urine (CREJ2U)

Sample size (n) = 69

Passing/Bablok[30]	Linear regression
y = 1.088x - 0.0452 mmol/L	y = 1.093x - 0.0846 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.787 and 49.1 mmol/L.

Creatinine values for human serum, plasma and urine 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).

Serum/plasma (CREJ2)

Sample size (n) = 86

Passing/Bablok[30]	Linear regression
y = 1.000x + 1.00 µmol/L	y = 0.997x + 2.37 µmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 25.5 and 2120 µmol/L.

Urine (CREJ2U)

Sample size (n) = 89

Passing/Bablok[30]	Linear regression
y = 0.983x - 0.0174 mmol/L	y = 0.984x - 0.0442 mmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 0.478 and 53.4 mmol/L.

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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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

CRP4

cobas®

Tina-quant C-Reactive Protein IV

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057591190	08057591500	Tina-quant C-Reactive Protein IV (500 tests)	System-ID 2050 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

11355279216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 20656	
20766321322	CRP T Control N (5 x 0.5 mL)	Code 20235	
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302	
11333127122	Precipath Protein (3 x 1 mL)	Code 20303	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

CRP4: ACN 20500

Intended use

Immunoturbidimetric assay for the in vitro quantitative determination of CRP in human serum and plasma on **cobas c** systems.

Summary

CRP measurements, performed with this assay in human serum or plasma, are used as aid in diagnosis, monitoring, prognosis, and management of suspected inflammatory disorders and associated diseases, acute infections and tissue injury.

C-reactive protein is the classic acute phase protein in inflammatory reactions.[1] It is synthesized by the liver and consists of 5 identical polypeptide chains that form a 5 membered ring having a molecular weight of 105000 daltons.[1,2,3,4] CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes.[2,3] Complexed CRP activates the classical complement pathway. The CRP response frequently precedes clinical symptoms, including fever.[1,3] After onset of an acute phase response the serum CRP concentration rises rapidly and extensively.[2,3,4] The increase begins within 6 to 12 hours and the peak value is reached within 24 to 48 hours.[1,3,5] Levels above 100 mg/L are associated with severe stimuli such as major trauma and severe infection (sepsis).[5] CRP response may be less pronounced in patients suffering from liver disease.[6]

CRP assays are used to detect systemic inflammatory processes (apart from certain types of inflammation such as systemic lupus erythematosus (SLE) and Colitis ulcerosa);[1,3,4,6] to assess treatment of bacterial infections with antibiotics;[1,4,6,7] to detect intrauterine infections with concomitant premature amniorrhexis;[4,6] to differentiate between active and inactive forms of disease with concurrent infection, e.g. in patients suffering from SLE or Colitis ulcerosa;[3,4,6] to therapeutically monitor rheumatic disease and assess anti-inflammatory therapy;[1,4,6] to determine the presence of post-operative complications at an early stage, such as infected wounds, thrombosis and pneumonia, and to distinguish between infection and bone marrow transplant rejection.[1,4,6]

Various assay methods are available for CRP determination, such as nephelometry and turbidimetry.[8,9] The Roche CRP assay is based on the principle of particle-enhanced immunological agglutination.

Test principle[10,8]

Particle-enhanced immunoturbidimetric assay

Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically.

Reagents - working solutions

R1 TRIS[a)] buffer with bovine serum albumin; preservatives

R3 Latex particles coated with anti-CRP (mouse) in glycine buffer; immunoglobulins (mouse); preservative

a) TRIS = Tris(hydroxymethyl)-aminomethane

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

CRP4

Tina-quant C-Reactive Protein IV

cobas®

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

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
Plasma: Li-heparin, K_2-EDTA, K_3-EDTA plasma

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.

Stability in serum and Li-heparin plasma:	2 weeks at 15-25 °C
	3 weeks at 2-8 °C
	12 months at -20 °C (± 5 °C)
Stability in K_2- and K_3-EDTA plasma:	1 day at 15-25 °C
	3 weeks at 2-8 °C
	12 months at -20 °C (± 5 °C)

Freeze only once.

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)	800/570 nm	
Reagent pipetting		Diluent (H_2O)
R1	98 µL	
R3	31 µL	16 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	2.6 µL	20 µL	60 µL
Increased	1.3 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: H_2O
	S2: Calibrator f.a.s. Proteins
Calibration mode	Non-linear
Calibration frequency	Full calibration - after reagent lot change - every 3 weeks on-board - every 6 months during shelf life - as required following quality control procedures

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

This method has been standardized against the certified reference material in human serum of the IRMM (Institute for Reference Materials and Measurements) ERM-DA474/IFCC.[11]

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 in the unit mg/L (nmol/L, mg/dL).

Conversion factors:	mg/L × 9.52 = nmol/L
	mg/L × 0.1 = mg/dL

Limitations - interference

Criterion: Recovery within ± 0.5 mg/L of initial values of samples ≤ 5.0 mg/L and within ± 10 % for samples > 5 mg/L.

Icterus:[12] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:[12] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 622 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[12] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 1200 IU/mL.

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 50 g/L (334 µmol/L) (simulated by human immunoglobulin G).

High-dose hook effect: No false result occurs up to a CRP concentration of 1200 mg/L.

In vitro tests were performed on commonly used pharmaceuticals. In addition, special pharmaceuticals were tested. Among them, the following substance caused interference:

Substance	No significant interference up to
Ticarcillin	225 mg/L

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

As with any assay employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample, which could cause falsely lowered results.

CRP4

Tina-quant C-Reactive Protein IV

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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

0.6-350 mg/L (5.7-3332 nmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.2 mg/L (1.9 nmol/L)
Limit of Detection	= 0.3 mg/L (2.9 nmol/L)
Limit of Quantitation	= 0.6 mg/L (5.7 nmol/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 C-reactive protein samples.

Expected values

Consensus reference interval for adults:[14] < 5 mg/L (< 47.6 nmol/L*)

*calculated by unit conversion factor

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) EP5-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.

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
CRP T Control N	3.33	0.0313	0.9
Precinorm Protein	9.72	0.0516	0.5
Precipath Protein	53.9	0.275	0.5
Human serum 1	1.11	0.0276	2.5
Human serum 2	4.09	0.0338	0.8
Human serum 3	82.9	0.474	0.6
Human serum 4	174	1.37	0.8
Human serum 5	305	2.10	0.7

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
CRP T Control N	3.33	0.0375	1.1
Precinorm Protein	9.72	0.0708	0.7
Precipath Protein	53.9	0.854	1.6
Human serum 1	1.11	0.0296	2.7
Human serum 2	4.09	0.0397	1.0
Human serum 3	82.9	1.61	1.9
Human serum 4	174	3.94	2.3
Human serum 5	305	5.79	1.9

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

CRP 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) = 157

Passing/Bablok[15]	Linear regression
y = 0.990x + 0.124 mg/L	y = 0.978x + 0.428 mg/L
τ = 0.995	r = 1.000

The sample concentrations were between 0.791 and 333 mg/L.

CRP 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) = 79

Passing/Bablok[15]	Linear regression
y = 0.976x - 0.0226 mg/L	y = 0.973x + 0.340 mg/L
τ = 0.989	r = 1.000

The sample concentrations were between 0.920 and 348 mg/L.

CRP 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) = 101

Passing/Bablok[15]	Linear regression
y = 1.013x + 0.0240 mg/L	y = 1.018x - 0.501 mg/L
τ = 0.992	r = 1.000

The sample concentrations were between 0.620 and 335 mg/L.

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.

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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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

GGT-2

cobas®

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057796190*	08057796500	γ-Glutamyltransferase ver.2 (400 tests)	System-ID 2060 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057796214*	08057796500	γ-Glutamyltransferase ver.2 (400 tests)	System-ID 2060 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

GGT2-I: ACN 20600: assay standardized against IFCC

GGT2-S: ACN 20601: assay standardized against Szasz

Intended use

In vitro test for the quantitative determination of γ-glutamyltransferase (GGT) in human serum and plasma on **cobas c** systems.

Summary

Measurements of γ-glutamyltransferase (GGT) performed with this assay in human serum and plasma are used in the diagnosis and monitoring of hepatobiliary diseases, as well as a screening test for occult alcoholism.

Mature GGT is a dimeric glycoprotein weighing 68 kDa. It is found in the kidneys, liver, pancreas, and intestine, with the highest abundance in renal tissue. However, the primary source of GGT activity in the serum is the liver.[1]

In clinical practice, GGT serum levels are typically measured alongside a full blood count, bilirubin, albumin, transaminases (ALT and AST), and alkaline phosphatases (ALP) as an initial investigation for potential liver disease.[2] GGT is considered one of the most reliable indicators for the development of liver disease.[3] Multiple guidelines recommend GGT testing as part of the diagnostic workup and monitoring for various liver diseases. Additionally, GGT serves as a well-established marker for alcohol-related liver disease and excessive alcohol consumption.[4,5,6,7,8,9,10] Increased GGT is observed as a result of obesity, excess alcohol consumption or may be induced by drugs, including phenobarbital and phenytoin.[1]

In 1969, Szasz published the first kinetic procedure for GGT in serum using γ-glutamyl-p-nitroanilide as substrate and glycylglycine as acceptor.[11] In order to circumvent the poor solubility of γ-glutamyl-p-nitroanilide, Persijn and van der Slik investigated various derivatives and found the water-soluble substrate L-γ-glutamyl-3-carboxy-4-nitroanilide to be superior in terms of stability and solubility.[12] The results correlate with those derived using the original substrate.

In 2002, the International Federation of Clinical Chemistry (IFCC) recommended the standardized method for determining GGT including optimization of substrate concentrations, employment of NaOH, glycylglycine buffer and sample start.[13,14] The GGT liquid reagent follows the formulation recommendation according to Szasz, but was optimized for performance and stability. The assay is optionally standardized against the original IFCC and Szasz methods. The performance claims and data presented here are independent from the standardization.

Test principle[15]

Enzymatic colorimetric assay

γ-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine.

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\text{GGT}}$ L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoate

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance photometrically.

Reagents - working solutions

R1 TRIS: 492 mmol/L, pH 8.25; glycylglycine: 492 mmol/L; preservative; additive

R3 L-γ-glutamyl-3-carboxy-4-nitroanilide: 22.5 mmol/L; acetate: 10 mmol/L, pH 4.5; stabilizer; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

GGT-2

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

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: Li-heparin and K_2-EDTA plasma

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.

Stability:[16,17]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

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)	700/415 nm		
Reagent pipetting		Diluent (H_2O)	
R1	19 µL	57 µL	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	–	–
Decreased	2.3 µL	10 µL	100 µL
Increased	2.3 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: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot 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 the original IFCC formulation (2002)[13] and against the GGT method published by Persijn and van der Slik (1976)[12], respectively.

Use the appropriate calibrator value for the corresponding application.

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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interferences

Criterion: Recovery within ± 4 U/L of initial values of samples ≤ 40 U/L and within ± 10 % for samples > 40 U/L.

Icterus:[18] No significant interference up to an I index of 50 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 855 µmol/L or 50 mg/dL and approximate unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis:[18] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[18] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[21]

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

3-1200 U/L (0.05-20.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 11.

GGT-2

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 3 U/L (0.05 µkat/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 activity 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 activity samples.

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

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

Expected values

U/L

Standardized against Szasz (Persijn, van der Slik)[22]

Men	8-61 U/L
Women	5-36 U/L

Standardized against IFCC

Reference Interval Study at 37 °C (corrected in 2005)[22,23]

Men (n = 216)	10-71 U/L
Women (n = 228)	6-42 U/L

Consensus values (IFCC)[24]

Men	< 60 U/L
Women	< 40 U/L

µkat/L

Standardized against Szasz (Persijn, van der Slik)[22,*]

Men	0.13-1.02 µkat/L
Women	0.08-0.60 µkat/L

Standardized against IFCC

Reference Interval Study at 37 °C (corrected in 2005)[22,23,*]

Men (n = 216)	0.17-1.19 µkat/L
Women (n = 228)	0.10-0.70 µkat/L

Consensus values (IFCC)[24]

Men	< 1.00 µkat/L
Women	< 0.67 µkat/L*

*calculated by unit conversion factor

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.

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	45.8	0.382	0.8
PCCC2[b)]	207	0.772	0.4
Human serum 1	8.57	0.449	5.2
Human serum 2	30.9	0.646	2.1
Human serum 3	62.7	0.679	1.1
Human serum 4	598	3.55	0.6
Human serum 5	1155	6.04	0.5

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	45.6	0.463	1.0
PCCC2[b)]	207	1.67	0.8
Human serum 1	7.97	0.420	5.3
Human serum 2	30.6	0.703	2.3
Human serum 3	62.7	0.708	1.1
Human serum 4	598	3.69	0.6
Human serum 5	1161	10.6	0.9

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

γ-glutamyltransferase 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) = 65

Passing/Bablok[25]	Linear regression
y = 1.014x - 1.98 U/L	y = 1.023x - 1.96 U/L
τ = 0.981	r = 0.999

The sample activities were between 4.81 and 941 U/L.

γ-glutamyltransferase 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) = 75

Passing/Bablok[25]	Linear regression
y = 1.010x + 1.44 U/L	y = 1.019x + 0.534 U/L
τ = 0.982	r = 1.000

The sample activities were between 3.10 and 1001 U/L.

γ-glutamyltransferase 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) = 77

Passing/Bablok[25]	Linear regression
y = 1.014x + 0.823 U/L	y = 1.013x + 1.03 U/L
τ = 0.962	r = 1.000

The sample concentrations were between 3.35 and 1157 U/L.

GGT-2

cobas®

γ-Glutamyltransferase ver.2 - Standardized against IFCC / Szasz

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
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+800 5505 6606

Roche

GLUC3

cobas®

Glucose HK Gen.3

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057800190*	08057800500	Glucose HK Gen.3 (3300 tests)	System-ID 2063 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057800214*	08057800500	Glucose HK Gen.3 (3300 tests)	System-ID 2063 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

GLUC3: ACN 20630 (Serum/plasma)

GLUC3U: ACN 20631 (Urine)

GLUC3C: ACN 20632 (CSF)

Intended use

In vitro test for the quantitative determination of glucose in human serum, plasma, urine and CSF on **cobas c** systems.

Summary

Glucose measurement in serum and plasma with this device can be used to aid in diagnosis and monitoring of hypo- and hyperglycemia, in the context of an altered carbohydrate metabolism state.

In urine, glucose measurement with this device can be used as an aid in diagnosing glycosuria in the context of altered carbohydrate metabolism states and/or kidney disease.

In CSF glucose measurement with this device can be used to aid in diagnosis and monitoring of central nervous system infections, such as meningitis and encephalitis of different etiologies.

Glucose is the major carbohydrate present in the peripheral blood.[1] Oxidation of glucose is the major source of cellular energy in the body.[2] Glucose derived from dietary sources is converted to glycogen for storage in the liver or to fatty acids for storage in adipose tissue. The concentration of glucose in blood is controlled within narrow limits by many hormones, the most important of which are produced by the pancreas.[1,2] The most frequent cause of hyperglycemia is diabetes mellitus resulting from a deficiency in insulin secretion or action.[1] Hypoglycemia is less frequently observed.[2] A variety of conditions may cause low blood glucose levels such as insulinoma, insulin induced hypoglycemia, or hypopituitarism.[2,3]

Under normal circumstances, almost all the glucose filtered by the glomerulus is reabsorbed in the proximal convoluted tubule.[4] In case of hyperglycemia, as occurs in diabetes mellitus, the tubular transport capacity of glucose is overwhelmed and glucose appears in the urine (glycosuria).[5] Furthermore, glycosuria occurs in the absence of hyperglycemia when the reabsorption of glucose by renal tubules is compromised.[4]

CSF glucose level and the corresponding plasma glucose ratio are usually altered (low) in certain types of central nervous system infections, such as bacterial and tuberculous meningitis. Whereas CSF glucose level and plasma glucose ratio concentration is typically normal during most viral CNS infections.[6,7] However, the spectrum of CSF glucose levels in bacterial meningitis is wide and there is substantial overlap with the findings in viral infection. Therefore, clinical evaluation and other laboratory tests are needed to guide treatment decisions besides the results of the CSF glucose and CSF plasma glucose ratio.[8]

Test principle

Enzymatic reference method with hexokinase.[9,10]

Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Reagents - working solutions

R1 MES buffer: 5.0 mmol/L, pH 6.0; Mg^{2+}: 24 mmol/L; ATP: ≥ 4.5 mmol/L; NADP: ≥ 7.0 mmol/L; preservative

R3 HEPES buffer: 200 mmol/L, pH 8.0; Mg^{2+}: 4 mmol/L; HK (yeast): ≥ 300 µkat/L; G-6-PDH (E. coli): ≥ 300 µkat/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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

P302 + P352 IF ON SKIN: Wash with plenty of water.

P332 + P313 If skin irritation occurs: Get medical advice/attention.

P337 + P313 If eye irritation persists: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.
Contact phone: all countries: +49-621-7590

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

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
Plasma: Li-heparin, K_2-EDTA, NaF/Na_2EDTA, KF/Na_2EDTA, NaF/K-Oxalate and NaF/citrate/Na_2-EDTA.

The stability of glucose in specimens is affected by storage temperature, bacterial contamination, and glycolysis. Plasma or serum samples without preservative (NaF) should be separated from the cells or clot within half an hour of being drawn. When blood is drawn and permitted to clot and to stand uncentrifuged at room temperature, the average decrease in serum glucose is ~ 7 % in 1 hour (0.28 to 0.56 mmol/L or 5 to 10 mg/dL). This decrease is the result of glycolysis. Glycolysis can be inhibited by collecting the specimen in fluoride tubes.[11]

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.

Stability:[10]	8 hours at 15-25 °C
	72 hours at 2-8 °C
Stability in fluoride plasma:[12]	3 days at 15-25 °C

Urine
Collect urine in a dark bottle. For 24-hour urine collections, glucose may be preserved by adding 5 mL of glacial acetic acid to the container before collection. Unpreserved urine samples may lose up to 40 % of their glucose after 24-hour storage at room temperature.[13] Therefore, keep samples on ice during collection.[10] If stabilizers are added to the sample, the sample index feature must not be used.

CSF
Cerebrospinal fluid may be contaminated with bacteria and often contains other cellular constituents. CSF samples should therefore be analyzed for glucose immediately or stored at 2-8 °C or -20 °C (± 5 °C).[13,10]

Freeze only once.

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, plasma, urine and CSF

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	21 µL	106 µL
R3	8 µL	15 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	3 µL	20 µL	60 µL
Increased	1.5 µL	–	–

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

Calibration

Application for serum/plasma (ACN 20630)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

Application for urine (ACN 20631)
Transfer of calibration from serum/plasma application (ACN 20630)

Application for CSF (ACN 20632)
Transfer of calibration from serum/plasma application (ACN 20630)

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

Traceability: This method has been standardized against ID/MS.

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.
CSF:	Quantitative CSF controls are recommended for routine quality control.

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 26 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 in the unit mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L x 18.02 = mg/dL
	mmol/L x 0.1802 = g/L

GLUC3

Glucose HK Gen.3

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Limitations - interference

Serum/plasma

Criterion: Recovery within ± 0.39 mmol/L of initial values of samples ≤ 3.9 mmol/L and within ± 10 % of samples > 3.9 mmol/L.

Icterus:[14] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[14] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[14] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[15,16]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[17]

Urine

Criterion: Recovery within ± 0.11 mmol/L of initial values of samples ≤ 1.1 mmol/L and within ± 10 % of samples > 1.1 mmol/L.

Urea: No significant interference from urea up to a concentration of 1800 mmol/L (10811 mg/dL).

Hemolysis: No significant interference up to an H index of 750 (approximate hemoglobin concentration: 466 µmol/L or 750 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16]

Tetracycline at therapeutic concentration gives falsely low results in urine samples.

CSF

Criterion: Recovery within ± 0.22 mmol/L of initial values of samples ≤ 2.2 mmol/L and within ± 10 % of samples > 2.2 mmol/L.

Icterus: No significant interference up to an I index of 60 for conjugated bilirubin (approximate conjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL.

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

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

NOTE: Glucose values achieved on some proficiency testing materials, when evaluated against a glucose oxidase-oxygen electrode comparison method, demonstrate an approximate 3 % positive bias on average.

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

Serum, plasma, urine and CSF

0.11-41.6 mmol/L (2-750 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.11 mmol/L (2 mg/dL)
Limit of Detection	= 0.11 mmol/L (2 mg/dL)
Limit of Quantitation	= 0.11 mmol/L (2 mg/dL)

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 glucose samples.

Expected values

mmol/L

Plasma[18]

Fasting	4.11-6.05 mmol/L

*Urine**[19]

1st morning urine	0.3-1.1 mmol/L
24-hour urine	0.3-0.96 mmol/L
	(average of 1350 mL urine/24 h)

* calculated by unit conversion factor

acc. to Tietz:[10]

Serum, plasma

Adults	4.11-5.89 mmol/L
60-90 years	4.56-6.38 mmol/L
> 90 years	4.16-6.72 mmol/L
Children	3.33-5.55 mmol/L
Neonates (1 day)	2.22-3.33 mmol/L
Neonates (> 1 day)	2.78-4.44 mmol/L

Urine

24-hour urine	< 2.78 mmol/24 h
Random urine	0.06-0.83 mmol/L

CSF

Children	3.33-4.44 mmol/L
Adults	2.22-3.89 mmol/L

mg/dL

Plasma[18]

Fasting	74-109 mg/dL

*Urine**[19]

1st morning urine	6-20 mg/dL
24-hour urine	6-17 mg/dL
	(average of 1350 mL urine/24 h)

* calculated by unit conversion factor

acc. to Tietz:[10]

Serum, plasma

Adults	74-106 mg/dL
60-90 years	82-115 mg/dL
> 90 years	75-121 mg/dL
Children	60-100 mg/dL
Neonates (1 day)	40-60 mg/dL
Neonates (> 1 day)	50-80 mg/dL

GLUC3

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Urine	
24-hour urine	< 2.78 mmol/24 h (< 0.5 g/24 h)
Random urine	1-15 mg/dL

CSF	
Children	60-80 mg/dL
Adults	40-70 mg/dL

CSF glucose values should be approximately 60 % of the plasma values and must always be compared with concurrently measured plasma values for adequate clinical interpretation.

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* *mmol/L*	*SD* *mmol/L*	*CV* *%*
PCCC1[a)]	5.61	0.0315	0.6
PCCC2[b)]	12.6	0.0523	0.4
Human serum 1	0.188	0.0174	9.2
Human serum 2	3.57	0.0181	0.5
Human serum 3	5.46	0.0233	0.4
Human serum 4	19.6	0.121	0.6
Human serum 5	38.6	0.188	0.5

Intermediate precision	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
PCCC1[a)]	5.61	0.0559	1.0
PCCC2[b)]	12.8	0.106	0.8
Human serum 1	0.188	0.0188	10.0
Human serum 2	3.57	0.0212	0.6
Human serum 3	5.46	0.0297	0.5
Human serum 4	19.6	0.136	0.7
Human serum 5	38.6	0.216	0.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
Control 1[c)]	1.09	0.0215	2.0
Control 2[c)]	16.4	0.0655	0.4
Human urine 1	0.227	0.0188	8.3
Human urine 2	0.733	0.0143	1.9
Human urine 3	4.10	0.0418	1.0
Human urine 4	22.0	0.182	0.8
Human urine 5	40.6	0.173	0.4

Intermediate precision	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
Control 1[c)]	1.09	0.0278	2.5
Control 2[c)]	16.4	0.122	0.7
Human urine 1	0.215	0.0183	8.5
Human urine 2	0.744	0.0180	2.4
Human urine 3	4.07	0.0478	1.2
Human urine 4	22.0	0.452	2.1
Human urine 5	40.4	0.344	0.8

CSF

Repeatability	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
Control 1[c)]	3.31	0.0119	0.4
Control 2[c)]	1.66	0.00970	0.6
Human CSF 1	0.273	0.00831	3.0
Human CSF 2	2.16	0.0180	0.8
Human CSF 3	3.81	0.0172	0.5
Human CSF 4	20.2	0.0824	0.4
Human CSF 5	39.9	0.193	0.5

Intermediate precision	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
Control 1[c)]	3.34	0.0163	0.5
Control 2[c)]	1.66	0.0109	0.7
Human CSF 1	0.273	0.00966	3.5
Human CSF 2	2.16	0.0212	1.0
Human CSF 3	3.81	0.0240	0.6
Human CSF 4	20.2	0.0994	0.5
Human CSF 5	39.9	0.230	0.6

c) commercially available control material

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

Glucose values for human serum, plasma, urine and CSF 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).

Serum/plasma

Sample size (n) = 74

Passing/Bablok[20]	Linear regression
y = 1.000x - 0.0200 mmol/L	y = 0.997x - 0.00454 mmol/L
τ = 0.987	r = 1.000

The sample concentrations were between 0.320 and 40.3 mmol/L.

Urine

Sample size (n) = 67

Passing/Bablok[20]	Linear regression
y = 0.995x - 0.0447 mmol/L	y = 0.995x - 0.0402 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 0.170 and 40.9 mmol/L.

CSF
Sample size (n) = 75

Passing/Bablok[20]	Linear regression
y = 1.000x + 0.00400 mmol/L	y = 1.001x + 0.0287 mmol/L
τ = 0.957	r = 0.999

The sample concentrations were between 0.200 and 40.8 mmol/L.

Glucose values for human serum, plasma, urine and CSF 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).

Serum/plasma
Sample size (n) = 69

Passing/Bablok[20]	Linear regression
y = 1.006x - 0.00351 mmol/L	y = 1.009x - 0.0366 mmol/L
τ = 0.977	r = 1.000

The sample concentrations were between 0.110 and 40.3 mmol/L.

Urine
Sample size (n) = 71

Passing/Bablok[20]	Linear regression
y = 1.012x - 0.0233 mmol/L	y = 1.022x - 0.0527 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 0.130 and 40.3 mmol/L.

CSF
Sample size (n) = 66

Passing/Bablok[20]	Linear regression
y = 1.019x + 0.0138 mmol/L	y = 1.020x + 0.0122 mmol/L
τ = 0.975	r = 1.000

The sample concentrations were between 0.290 and 39.4 mmol/L.

Glucose values for human serum, plasma, urine and CSF 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).

Serum/plasma
Sample size (n) = 74

Passing/Bablok[20]	Linear regression
y = 0.995x - 0.00537 mmol/L	y = 0.999x - 0.0120 mmol/L
τ = 0.990	r = 1.000

The sample concentrations were between 0.200 and 40.2 mmol/L.

Urine
Sample size (n) = 67

Passing/Bablok[20]	Linear regression
y = 0.998x + 0.0116 mmol/L	y = 1.001x + 0.0119 mmol/L
τ = 0.950	r = 1.000

The sample concentrations were between 0.113 and 40.5 mmol/L.

CSF
Sample size (n) = 75

Passing/Bablok[20]	Linear regression
y = 0.987x - 0.0130 mmol/L	y = 0.989x - 0.0144 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.154 and 40.5 mmol/L.

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume for reconstitution

GLUC3

Glucose HK Gen.3

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
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HDLC4

cobas®

HDL-Cholesterol Gen.4

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057877190	08057877500	HDL-Cholesterol Gen.4 (700 tests)	System-ID 2071 002	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057877214*	08057877500	HDL-Cholesterol Gen.4 (700 tests)	System-ID 2071 002	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

12172623122	Calibrator f.a.s. Lipids (3 x 1 mL)	Code 20424	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

HDLC4: ACN 20710

Intended use

In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on **cobas c** systems.

Summary

Measurements of HDL-cholesterol, performed with this assay in human serum or plasma, are used for screening, aid in diagnosis and monitoring of dyslipidaemias as well as for assessment of cardiovascular risk such as in ASCVD and CHD.

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. In the liver, cholesterol is transformed to bile acids which are then excreted into the intestine via the biliary tract.
Monitoring of HDL-cholesterol in serum or plasma is of clinical relevance as the HDL-cholesterol concentration is important in the assessment of atherosclerotic cardiovascular risk (ASCVD).[1] Elevated HDL-cholesterol concentrations protect against coronary heart disease (CHD), whereas reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.[1]

Two cholesterol related variables that are predictive of cardiovascular disease (CVD) have emerged. These are non-HDL-cholesterol[2,3,4] (= total cholesterol - HDL-cholesterol) and the rate of cholesterol transfer from the macrophages to HDL, also described as cholesterol efflux capacity.[5]
Whereas both cholesterol and HDL-cholesterol can be readily determined with high accuracy, currently, non-HDL-cholesterol appears to be best suited for patient management.[2,3,4]

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation (reference method in combination with cholesterol measurement by the Abell-Kendall method), electrophoresis, high performance liquid chromatography (HPLC), precipitation, and direct methods.[6,7] Of these, the direct methods are used routinely. Roche HDLC4 is also a direct method. The automated HDLC4 assay uses detergents, cholesterol esterase (CHER), cholesterol oxidase (CHOD) and peroxidase to form a colored pigment that is measured optically.[8,9]

The HDLC4 assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for precision and accuracy.[10,11]

Test principle[8,9]

Homogeneous enzymatic colorimetric test.

Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of CHER and CHOD towards non-HDL lipoproteins is blocked.
Finally only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.
Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER.

$$\text{HDL-cholesterol esters} + H_2O \xrightarrow{\text{CHER}} \text{HDL-cholesterol} + \text{RCOOH}$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$\text{HDL-cholesterol} + O_2 \xrightarrow{\text{CHOD}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and EMSE[a)] to form a dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

$$2\ H_2O_2 + \text{4-amino-antipyrine} + \text{EMSE} + H^+ \xrightarrow{\text{Peroxidase}} \text{colored pigment} + 5\ H_2O$$

a) N-ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

Reagents - working solutions

R1 TAPSO[b)] buffer: 62.1 mmol/L, pH 7.77; polyanion: 1.25 g/L; EMSE: 1.08 mmol/L; ascorbate oxidase (cucurbita): ≥ 50 µkat/L; peroxidase (horseradish): ≥ 166.7 µkat/L; detergent; BSA: 2.0 g/L; preservative

R3 Bis-Tris[c)] buffer: 20.1 mmol/L, pH 6.70; cholesterol esterase (microorganism): ≥ 7.5 µkat/L; cholesterol oxidase (recombinant E. coli): ≥ 7.17 µkat/L; cholesterol oxidase (microorganism): ≥ 76.7 µkat/L; peroxidase (horseradish): ≥ 333 µkat/L; 4-amino-antipyrine: 1.48 mmol/L; BSA: 3.0 g/L; detergents; preservative

b) 2-Hydroxy-N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid

c) Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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

The intrinsic color of the reagent does not interfere with the test.

HDLC4

HDL-Cholesterol Gen.4

cobas®

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

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.
Plasma: Li-heparin, K_2- and K_3-EDTA plasma.

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.

Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

Fasting and non-fasting samples can be used.[12,13]

Stability in serum:	72 hours at 15-25 °C[13]
	7 days at 2-8 °C[13]
	12 months at -20 °C (± 5 °C)[14]
	24 months at -70 °C (± 5 °C)[15]

Freeze only once.

Stability in Li-heparin, K_2- and K_3-EDTA plasma:	72 hours at 15-25 °C[13]
	7 days at 2-8 °C[13]
	3 months at -20 °C (± 5 °C) °C[13]
	18 months at -70 °C (± 5 °C)[13]
	18 months at -80 °C (± 5 °C)[16]

Freeze only once.

It is reported that EDTA stabilizes lipoproteins.[17]

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

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)	700/600 nm	
Reagent pipetting		Diluent (H_2O)
R1	80 µL	–
R3	27 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.6 µL	–	–
Decreased	8.0 µL	10 µL	90 µL
Increased	1.6 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: H_2O
	S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 the designated CDC reference method (ultracentrifugation method).[10] The standardization meets the requirements of the “HDL Cholesterol Method Evaluation Protocol for Manufacturers” of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.

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 in the unit mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L x 38.66 = mg/dL
	mmol/L x 0.3866 = g/L

Limitations - interference[18]

Criterion: Recovery within ±0.1 mmol/L of initial values of samples ≤ 1 mmol/L (38.7 mg/dL) and within ±10 % for samples > 1 mmol/L.

Icterus:[19] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[19] No significant interference up to an H index of 1200 (approximate hemoglobin concentration: 745 µmol/L or 1200 mg/dL).

Lipemia (Intralipid):[19] No significant interference up to an L index of 2000. No significant interference from native triglycerides up to 13.7 mmol/L or 1200 mg/dL. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other: Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 2.84 mmol/L (50 mg/dL).

Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol result may significantly differ from the DCM (designated comparison method) result due to the presence of lipoproteins with abnormal lipid distribution.[20]

Drugs: No interference was found at therapeutic concentrations using common drug panels.[21,22]

Statins (Simvastatin) and fibrates (Bezafibrate) tested at therapeutic concentration ranges did not interfere.

N-acetylcysteine: No significant interference from N-acetylcysteine up to a concentration of 2.76 mmol/L (450 mg/L).

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-acetylcysteine at the therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low HDL-cholesterol results.

Metamizole: Venipuncture should be performed prior to the administration of metamizole. Venipuncture immediately after or during the administration of metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[23]

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

0.08-3.88 mmol/L (3.09-150 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.08 mmol/L (3.09 mg/dL)
Limit of Detection	= 0.08 mmol/L (3.09 mg/dL)
Limit of Quantitation	= 0.08 mmol/L (3.09 mg/dL)

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 precision of ≤ 30 % CV. It has been determined using low concentration HDL-cholesterol samples.

Expected values

mmol/L

	No risk	Moderate risk	High risk
Females[7,24,25]	> 1.68 mmol/L	1.15-1.68 mmol/L	< 1.15 mmol/L
Males[7,24,25]	> 1.45 mmol/L	0.90-1.45 mmol/L	< 0.90 mmol/L

mg/dL

	No risk	Moderate risk	High risk
Females[7,24,25]	> 65 mg/dL	45-65 mg/dL	< 45 mg/dL
Males[7,24,25]	> 55 mg/dL	35-55 mg/dL	< 35 mg/dL

National Cholesterol Education Program (NCEP) guidelines:[26]

< 40 mg/dL: Low HDL-cholesterol (major risk factor for CHD)

≥ 60 mg/dL: High HDL-cholesterol ("negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercise, hormones, sex and age.

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

National Cholesterol Education Program (NCEP) guidelines are based on serum values. When classifying patients, serum or serum equivalent values should be used. Therefore the NCEP recommends using a factor of 1.03 to convert EDTA plasma values to serum values. A later study found EDTA plasma concentrations to be 4.7 % lower than those in serum.[27] To comply with the 1998 NCEP goal of a bias < 5 % it is recommended that each laboratory validates this conversion factor and enters it into the test parameters for HDL-cholesterol.[28]

Treatment goals for non-HDL-cholesterol have been proposed:[2]

	NCEP ATP III	ADA/AHA Guidelines for patients with increased cardiometabolic risk
Optional goal for very-high/highest risk patients (known CVD, diabetes with elevated risk)	< 3.37 mmol/L (< 130 mg/dL)	< 2.59 mmol/L (< 100 mg/dL)
Optional goal for those with established cardiovascular disease and multiple major risk factors	< 2.59 mmol/L (< 100 mg/dL)	
Optional goal for high-risk patients, CHD-risk-equivalent (Framingham 10-year risk score > 20 %/10 years, diabetes without other major risk factors)	< 3.37 mmol/L (< 130 mg/dL)	< 3.37 mmol/L (< 130 mg/dL)
Optional goal for moderately-high/intermediate risk patients (≥ 2 major CVD risk factors, Framingham 10-year risk score from 10-20 %)	< 4.14 mmol/L (< 160 mg/dL)	< 3.37 mmol/L (< 130 mg/dL)
Optional goal for high-risk patients, CHD-risk-equivalent (Framingham 10-year risk score > 20 %/10 years, diabetes without other major risk factors)	< 3.37 mmol/L (< 130 mg/dL)	

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.

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[d)]	0.764	0.00290	0.4
PCCC2[e)]	1.45	0.00690	0.5

Human serum 1	0.148	0.00177	1.2
Human serum 2	1.07	0.00512	0.5
Human serum 3	1.49	0.00673	0.5
Human serum 4	1.92	0.00715	0.4
Human serum 5	3.53	0.0152	0.4
Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[d)]	0.760	0.00630	0.8
PCCC2[e)]	1.44	0.00974	0.7
Human serum 1	0.148	0.00229	1.5
Human serum 2	1.07	0.00708	0.7
Human serum 3	1.49	0.0105	0.7
Human serum 4	1.92	0.0145	0.8
Human serum 5	3.54	0.0249	0.7

d) PreciControl ClinChem Multi 1

e) PreciControl ClinChem Multi 2

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

HDL-cholesterol 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) = 70

Passing/Bablok[29]	Linear regression
y = 1.001x - 0.0175 mmol/L	y = 1.012x - 0.0274 mmol/L
τ = 0.976	r = 1.000

The sample concentrations were between 0.110 and 3.57 mmol/L.

HDL-cholesterol 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) = 70

Passing/Bablok[29]	Linear regression
y = 1.011x - 0.0242 mmol/L	y = 1.028x - 0.0389 mmol/L
τ = 0.977	r = 1.000

The sample concentrations were between 0.110 and 3.57 mmol/L.

HDL-cholesterol 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) = 75

Passing/Bablok[29]	Linear regression
y = 1.000x - 0.0200 mmol/L	y = 1.000x - 0.0159 mmol/L
τ = 0.994	r = 1.000

The sample concentrations were between 0.115 and 3.67 mmol/L.

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

IRON2

cobas®

Iron Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057931190*	08057931500	Iron Gen.2 (700 tests)	System-ID 2077 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057931214*	08057931500	Iron Gen.2 (700 tests)	System-ID 2077 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

IRON2: ACN 20770

Intended use

In vitro test for the quantitative determination of iron in human serum and plasma on **cobas c** systems.

Summary

Iron measurements performed with this assay in human serum and plasma are used as an aid in diagnosis and monitoring of iron deficiency and iron overload disorders.

Iron is essential for many metabolic and biochemical processes. Similar to other micronutrients in the human body, iron is supplied with food. Ingested iron is mainly absorbed in the form of Fe^{2+} in the duodenum and proximal jejunum. The trivalent form and the heme-bound Fe^{3+}-component of iron in food has to be reduced by duodenal cytochrome B. About 1-2 mg of iron is absorbed and lost daily. Upon reaching the mucosal cells, Fe^{2+} ions become bound to transport proteins. In the cellular phase iron is either stored in cellular ferritin or transported to the circulation. Iron export into the circulation requires Fe^{2+} oxidation to Fe^{3+} by hephaestin (on cellular membrane) or ceruloplasmin (in the circulation), for loading onto transferrin. Circulating Fe ions are transported by transferrin-iron complexes. A maximum of 2 Fe^{3+} ions per protein molecule can be transported.[1]

Serum iron fluctuates with dietary intake and normal diurnal variation. Clinically, dysregulation of serum/plasma iron levels can be divided into iron deficiency and iron overload.[1,2] Iron deficiency disorders can be due to increased demands (e.g. growth, pregnancy), limited external supply (e.g. malnutrition, inappropriate diet, malabsorption), increased loss (e.g. hemorrhage, hemodialysis, blood donation), or other conditions, such as chronic kidney disease resulting in renal anemia, inflammatory bowel disease, heart failure, obesity, bone marrow disease.[2,3] Iron deficiency occurs in several stages, defined by the extent of depletion, first of iron stores and then of iron available for hemoglobin synthesis. In the first stage, iron stores can be completely depleted without causing anemia. Further iron loss causes anemia (iron deficiency anemia, IDA), which is initially normocytic, with a normal absolute reticulocyte count. Deeper deficiency results in classic anemia findings with hypochromic (low mean corpuscular hemoglobin) and microcytic (low mean corpuscular volume) red blood cells.[3,4,5] Another type of anemia is macrocytic anemia (elevated mean corpuscular volume), which is not directly due to iron deficiency but is rather related to other causes, such as vitamin B12 and folate deficiency, bone marrow disorders (myelodysplasia), use of certain medications, alcohol abuse, liver disease, marked reticulocytosis, and hypothyroidism. Iron measurements can help define different causes of anemia.[6,7]

Iron overload disorders normally result in increased serum/plasma iron concentration, and can be due to a number of underlying conditions, most commonly hereditary haemochromatosis (excess iron derived from increased gastrointestinal absorption due to inactivating mutations in components of the hepcidin pathway) and thalassemia (increased concentrations of iron mainly caused from regular red blood cell transfusions and to a lesser extent by increased iron absorption).[2,8]

Numerous photometric methods have been described for the determination of iron. All have the following in common:

- Liberation of Fe^{3+} ions from the transferrin complex using acids or detergents.
- Reduction of Fe^{3+} ions to Fe^{2+} ions.
- Reaction of the Fe^{2+} ions to give a colored complex.[1]

The method described here is based on the FerroZine method without deproteinization.

Test principle

Colorimetric assay.

$$\text{Transferrin-Fe-complex} \xrightarrow{\text{pH} < 2.0} \text{apotransferrin} + Fe^{3+}$$

$$Fe^{3+} \xrightarrow{\text{ascorbate}} Fe^{2+}$$

$$Fe^{2+} + \text{FerroZine} \longrightarrow \text{colored complex}$$

Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe^{3+} ions to Fe^{2+} ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically.

Reagents - working solutions

R1	Citric acid: 200 mmol/L; thiourea: 115 mmol/L; detergent
R3	Sodium ascorbate: 150 mmol/L; FerroZine: 6 mmol/L; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

IRON2

Iron Gen.2

Danger

H318 Causes serious eye damage.

Prevention:

P280 Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Hazardous components:

- Poly(oxy-1,2-ethanediyl), .alpha.-isotridecyl-.omega.-hydroxy-

EUH 208 Contains DIAZOLIDINYL UREA. May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

When removing the **cobas c** pack from the instrument during use, please immediately store at 2-8 °C.

Do not shake the **cobas c** pack to avoid foaming.

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.

Plasma: Li-heparin plasma. Do not use EDTA or oxalate plasma.

Separate serum or plasma from the clot or cells within 1 hour.

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.

Stability:[9,10]	7 days at 15-25 °C
	3 weeks at 2-8 °C
	several years at -20 °C (± 5 °C)

Freeze only once.

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)	700/570 nm		
Reagent pipetting		Diluent (H_2O)	
R1	75 µL	–	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	6.4 µL	–	–
Decreased	9.0 µL	25 µL	50 µL
Increased	6.4 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: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change
	1-point recalibration using S1 - after **cobas c** pack green change - every 7 days on-board

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

Traceability: This method has been standardized against a primary reference material (SRM 937).

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 in the unit µmol/L (µg/dL, mg/L).

Conversion factors:	µmol/L x 5.59 = µg/dL
	µmol/L x 0.0559 = mg/L

Limitations - interference

Criterion: Recovery within ± 2.7 µmol/L of initial values of samples ≤ 26.9 µmol/L and within ± 10 % for samples > 26.9 µmol/L.

Icterus:[11] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[11] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 125 µmol/L or 200 mg/dL). Higher hemoglobin concentrations lead to artificially increased values due to contamination of the sample with hemoglobin-bound iron.

Lipemia (Intralipid):[11] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12, 13]

In patients treated with iron supplements or metal-binding drugs, the drug-bound iron may not properly react in the test, resulting in artificially low values.

In the presence of high ferritin concentrations > 1200 µg/L the assumption that serum iron is almost completely bound to transferrin is not valid anymore. Therefore, such iron results should not be used to calculate Total Iron Binding Capacity (TIBC) or percent transferrin saturation (% SAT).[14]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

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

0.90-179 µmol/L (5.00-1000 µg/dL, 0.05-10.0 mg/L)

Determine samples having higher concentrations via the rerun function. For samples with higher concentrations, the rerun function decreases the sample volume by a factor of 2.1. The results are automatically multiplied by this factor.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.9 µmol/L (5.03 µg/dL)
Limit of Detection	= 0.9 µmol/L (5.03 µg/dL)
Limit of Quantitation	= 0.9 µmol/L (5.03 µg/dL)

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 iron samples.

Expected values[16]

µmol/L

Adults:	5.83-34.5 µmol/L

µg/dL

Adults:	33-193 µg/dL

The concentration of iron in serum/plasma is dependent on ingestion of iron and is subject to circadian variations.[17]

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.

Repeatability	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[a)]	18.6	0.111	0.6
PCCC2[b)]	41.4	0.163	0.4
Human serum 1	2.37	0.0817	3.4
Human serum 2	6.01	0.0830	1.4
Human serum 3	35.1	0.135	0.4
Human serum 4	89.2	0.307	0.3
Human serum 5	158	0.655	0.4

Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[a)]	18.6	0.212	1.1
PCCC2[b)]	41.6	0.369	0.9
Human serum 1	2.32	0.120	5.2
Human serum 2	5.95	0.149	2.5
Human serum 3	35.1	0.187	0.5
Human serum 4	89.2	0.337	0.4
Human serum 5	158	0.673	0.4

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

Iron 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) = 74

Passing/Bablok[18]	Linear regression
y = 1.004x + 0.0354 µmol/L	y = 1.003x + 0.00110 µmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 1.20 and 169 µmol/L.

Iron 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) = 98

Passing/Bablok[18]	Linear regression
y = 1.011x - 0.0750 µmol/L	y = 1.011x - 0.0772 µmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 1.72 and 172 µmol/L.

IRON2

Iron Gen.2

Iron 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) = 75

Passing/Bablok[18]	Linear regression
y = 1.000x + 0.0000 µmol/L	y = 1.001x - 0.0494 µmol/L
τ = 0.998	r = 1.000

The sample concentrations were between 2.55 and 176 µmol/L.

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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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K Electrode

cobas®

Potassium

Order information

REF	CONTENT	Analyzer(s) on which the electrode can be used
10825441001	K Electrode 1 (electrode)	**cobas c** 311 analyzer **cobas** 6000 analyzer series: **cobas c** 501 module **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module **cobas pure** integrated solutions: **cobas c** 303 analytical unit **cobas pro** integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Materials required (but not provided):

03149501001	REF Electrode (1 electrode)	
11360981216	ISE Reference Electrolyte (5 x 300 mL) ①②	
10820652216	ISE Reference Electrolyte (1 x 500 mL) ③④	
08392013190	ISE Reference Electrolyte (2 x 2000 mL) ⑤⑥	
04522320190	ISE Internal Standard Gen.2 (5 x 600 mL) ①②	
04880455190	ISE Internal Standard Gen.2 (2 x 2000 mL) ③④⑤	
09137742190	ISE Internal Standard Gen.2 conc. (1 x 510 mL) ⑥	
05979854190	Internal Standard Insert - ISE (Set of 20) ①②	
04522630190	ISE Diluent Gen.2 (5 x 300 mL) ①②	
04880480190	ISE Diluent Gen.2 (2 x 2000 mL) ③④⑤	
11298500316	ISE Cleaning Solution (5 x 100 mL)	
20763071122	ISE Deproteinizer (6 x 21 mL) ④⑤⑥	
03110435180	Deproteinizer (1 x 125 mL) ⑥	
04663632190	Activator (9 x 12 mL)	
11183974216	ISE Standard Low (10 x 3 mL)	Code 20502
11183982216	ISE Standard High (10 x 3 mL)	Codes 20503, 20763
12149435122	Precinorm U Plus (10 x 3 mL)	Code 20300
12149443122	Precipath U Plus (10 x 3 mL)	Code 20301
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392

ISE reagents on:

① **cobas c** 311 analyzer

② **cobas** 6000 analyzer series: **cobas c** 501 module

③ **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

④ **cobas pure** integrated solutions: **cobas c** 303 analytical unit

⑤ **cobas pro** integrated solutions: **cobas pro** ISE analytical unit

⑥ **cobas pro** integrated solutions: **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

English

System information

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE K	**ISE K-U**	**ISE K-P**	**ISE K-S**
cobas c 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module	990	990	---	---

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE K	**ISE K-U**	**ISE K-P**	**ISE K-S**
cobas c 303 analytical unit, **cobas pro** ISE analytical unit	29080	29081	29082	29083

K Electrode

cobas®

Potassium

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	K	**K-U**	**K-P**	**K-S**
cobas ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit	29240	29241	29242	29243

Intended use

The K Electrode is a device intended for the in-vitro quantitative determination of potassium in human origin serum, plasma and urine.

Summary[1]

Electrolytes are involved in most major metabolic functions in the body.

Potassium is the major intracellular cation and is critical to neural and muscle cell activity.

Some causes of decreased potassium levels include reduced intake of dietary potassium or excessive loss of potassium from the body due to diarrhea, prolonged vomiting, or increased kidney excretion. Increased potassium levels may be caused by dehydration or shock, severe burns, diabetic ketoacidosis, and retention of potassium by the kidney.

Test principle

Ion-selective electrode, using automatically diluted serum/plasma or urine specimens. The potassium electrode is based on a neutral carrier (Valinomycin).[2]

Calculation

The equation given below is used for the calculation of sample and/or QC results:

$$C_S = C_{IS} \times 10^{\frac{E_S - E_{IS}}{\pm S}}$$

Where:

C_S	concentration of the ion in the sample
C_{IS}	concentration of the ion in the ISE Internal Standard
E_S	EMF of the sample
E_{IS}	EMF of the ISE Internal Standard
S	Slope of the electrode

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration.

Precautions and warnings

For in vitro diagnostic use for trained laboratory technicians.

Warning

- Samples containing material of human origin are potentially infectious. Wear personal protective equipment when replacing or installing electrodes at analyzers. If any biohazardous material is spilled, wipe it up immediately and apply a disinfectant.
- If sample or waste contacts with your skin, wash the affected area immediately with soap and water, then apply a disinfectant. Consult a physician.
- When disposing of used electrodes, treat them as biohazardous.

Caution

- Do not use electrodes after the shelf life or on-board stability period has expired. Otherwise, it may lead to unstable sodium, potassium, and chloride results due to the unstable potential reading of electrodes.
- Do not use hemolyzed samples because of falsely higher potassium results. Potassium concentration in erythrocytes is 25 times higher than in normal plasma.
- Perform electrode flow path cleaning as stated in the Instructions for Use for applicable analyzers, at the end of a daily sample run. Improper electrode flow path cleaning may cause unstable reading of electrodes and it results in calibration failures.

As with any diagnostic test procedure, results should be interpreted taking all other test results and the clinical status of the patient into consideration.

In addition, pay attention to all precautions and warnings listed in the operator's manual of the analyzer.

NOTE: Boric acid (CAS Registry No. 10043-35-3) is contained in the gel solution inside the electrode at 0.2 % of the total weight as a preservative.

Storage and stability

Store at 7-40 °C.

See labels for expiration dates.

On-board stability

After installation the electrode is stable for the following time period:
2 months or 9000 tests, whichever comes first.

The electrodes should be replaced after this time period has expired. For replacement refer to instructions in the operator's manual of the applicable analyzers.

NOTE: When replacing the electrode in **cobas pro** or **cobas pure**, the user should scan the barcode affixed on the rear side of the package instead of the barcode placed on the product's label.

Slope range 50 to 68 mV/dec

NOTE: The slope ranges for newly installed electrodes should be in the upper half of the recommended electrode slope range.

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.

It is important to follow tube manufacturers recommended procedures at and after blood collection.

Separate from cells if analysis is not performed within 2 hours.[3]

Serum

Use serum free of hemolysis and gross lipemia, collected by standard venipuncture technique.

As described in the literature, potassium values in serum are increased compared to plasma. Serum potassium is released from platelets during clotting. The higher the platelet count, the greater the error.[4]

Plasma is preferable to serum as sample material for potassium determinations.

CAUTION: Serum separator tubes have to be used in accordance with the tube manufacturer's recommended procedures. If these procedures are not considered, it is possible to coat the sample probe with gel (interfering with proper sample level detection), or even to aspirate gel into the ISE system (resulting in a clogged system).

Plasma: Lithium heparin plasma

For potassium determinations, plasma is the specimen of choice.

CAUTION: Inadequate mixing of plasma tubes can cause introduction of fibrin microclots into and subsequent clogging of the ISE.

NOTE: For certain types of hematological neoplasias, (severe) pseudohyperkalemia using lithium heparin samples has been reported.[5,6,7]

NOTE: It is strongly recommended to avoid silicone-type gels, due to risk of silicon oil contaminations. In addition, tubes that exhibit a layer of clear liquid, which rises to the top of the serum after centrifugation, should not be used, in order to prevent coating the sample probes and interfering with ISE system. It is possible to clog the sample probes or the ISE tubing with gel or clots if these precautions are not taken.

Urine: Collect 24-hour urine without addition of preservatives and/or stabilizers. Store refrigerated during collection.

NOTE: Each laboratory should establish guidelines for determining acceptability of specimens and the corrective action to be taken if a specimen is considered unacceptable. Compile a laboratory-specific guideline.

Sample stability (serum, plasma): [8]

14 days at 15-25 °C

14 days at 2-8 °C

stable at (-15)-(-25) °C

K Electrode

Potassium

up to 10 freeze-thaw cycles possible.[9]

Sample stability (urine):[8,10]

14 days at 15-25 °C

stable at (-15)-(-25) °C

up to 6 freeze-thaw cycles possible.[11]

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

Sample stability claims were established by experimental data by the manufacturer or based on reference literature[8] and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Order information" section

Materials required (but not provided)

See "Order information" section

General laboratory equipment

Application for serum, plasma and urine

Test definition

Serum/plasma

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 1.5-10 mmol/L

Analysis of potassium on ISE analytical units listed with serum and plasma specimens should yield a linear relationship from 1.5-10 mmol/L with a deviation from the linear line of less than 5 %.

The sample volumes given above under "Normal" are for samples, calibrators, and quality controls.

Urine

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
Decreased	6.5 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module		
Normal	10 µL	450 µL / ISE Diluent
Increased	not applicable	not applicable
cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
Decreased	10 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water
Decreased	10 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 3-100 mmol/L

Analysis of potassium on ISE analytical units listed with urine specimens should yield a linear relationship from 3-100 mmol/L with a deviation from the linear line of less than 10 %.

Determine samples having higher concentrations via the rerun function. Dilution of samples via rerun function is a 1:46 dilution. Results from samples diluted using the rerun function are automatically multiplied by the dilution factor.

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit* for urine samples with decreased sample volume (Rerun): 101-150 mmol/L.

Analysis of potassium on ISE analytical units listed with urine specimens should yield a linear relationship from 101-150 mmol/L with a deviation from the linear line of less than 10 %.

The sample volumes given above under "Normal" are for samples, calibrators, and quality controls.

Measuring range on *cobas 8000 ISE 900 / 1800 module:* 3-100 mmol/L

Analysis of potassium on **cobas** 8000 ISE 900 / 1800 module with urine specimens should yield a linear relationship from 3-100 mmol/L with a deviation from the linear line of less than 10 %.

A diluton of samples via rerun function is not applicable for urinary potassium.

The sample volumes given above under "Normal" are for samples and quality controls.

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

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 1 mmol/L
Limit of Detection	= 1 mmol/L
Limit of Quantitation	= 1.5 mmol/L
Limit of Quantitation for urinary potassium on **cobas** 8000 ISE 900 / 1800 module	= 3.0 mmol/L

The Limit of Blank, the Limit of Detection and the 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 30 %. It has been determined using low concentration potassium samples.

Values below Limit of Quantitation are not reliable due to possible higher uncertainty.

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.

Calibration

Calibration requires the following calibrators: ISE Standard Low (S1), ISE Standard High (S2), and ISE Standard High (S3).

K Electrode

cobas®

Potassium

The slope of the calibration curve is calculated from Standards 1 and 2. ISE Internal Standard / ISE Internal Standard conc. is measured to provide E_{IS} for all measurements. Refer to the operator's manual of the analyzer for detailed calibration instructions.

Traceability: ISE Standard Low and ISE Standard High are prepared gravimetrically from highly purified inorganic salts.

Purity of these salts has been certified by argentometric titration, acidimetric titration or perchloric acid titration.

Calibration frequency

Calibration

- every 24 hours
- after ISE washing and maintenance
- after changing the reagent bottle ①
- after changing ISE Reference Electrolyte and/or Internal Standard conc. (depending on AutoCal settings) ②
- after replacing any electrode
- as required following quality control procedures

ISE reagents on:

① **cobas c** 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module, **cobas c** 303 analytical unit, **cobas pro** ISE analytical unit

② **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Refer to the operator's manual for a detailed description of the Calibration/AutoCal function.

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
	Precinorm U Plus, Precipath U Plus
Urine:	Quantitative urine controls are recommended for routine quality control.

Quality controls should be performed daily and after every additional calibration.

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.

Refer to appropriate value sheets/package inserts for additional information.

Traceability: Each Roche Diagnostics control listed above has been standardized against ISE Standard Low and ISE Standard High.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Hemolysis - serum/plasma

Do not use hemolyzed samples.

Potassium concentration in erythrocytes is 25 times higher than in normal plasma. The level of interference may be variable depending on the exact content of erythrocytes.

An H index of ≤ 20 equals an increase of the potassium concentration of ≤ 0.1 mmol/L.[12]

Hemolysis - urine

Hemolysis:[13] No significant interference up to a hemoglobin concentration of 12.4 µmol/L or 20 mg/dL.

Icterus - serum/plasma

Icterus:[13] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Lipemia - serum/plasma

Lipemia (Intralipid, SMOFlipid):[13] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

NOTE: Grossly lipemic specimens should be cleared by ultracentrifugation.[14]

Drugs

The following drugs have been tested and caused no significant interference when added to aliquots of pooled normal human serum up to the indicated concentration.

Serum/plasma

Acetaminophen (Paracetamol)	200 mg/L
Acetylsalicylic acid	1000 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Cefoxitin	2500 mg/L
Cyclosporine	5 mg/L
Doxycyclin	50 mg/L
Heparin	5000 IU/L
Ibuprofen	500 mg/L
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
N-Acetylcysteine	1660 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Theophylline	100 mg/L

Urine

Acetaminophen (Paracetamol)	3000 mg/L
Ascorbic acid	4000 mg/L
Cefoxitin	12000 mg/L
Gentamycine sulfate	400 mg/L
Ibuprofen	4000 mg/L
Levodopa	1000 mg/L
Methyldopa	2000 mg/L
N-Acetylcysteine	10 mg/L
Ofloxacine	900 mg/L
Phenazopyridine	300 mg/L
Salicyluric acid	6000 mg/L
Tetracycline	300 mg/L

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.

Expected values[15]

Serum	Infant	4.1-5.3 mmol/L
	Child	3.4-4.7 mmol/L
	Adult	3.5-5.1 mmol/L

K Electrode

Potassium

Plasma	M	3.5-4.5 mmol/L
	F	3.4-4.4 mmol/L

Plasma potassium levels are reported to be lower than serum levels.

Urine 24 h	6-10 y, M	17-54 mmol/24 h
	6-10 y, F	8-37 mmol/24 h
	10-14 y, M	22-57 mmol/24 h
	10-14 y, F	18-58 mmol/24 h
	Adult	25-125 mmol/24 h

The urinary excretion of potassium varies significantly with dietary intake. The values given here are typical of people on an average diet.

NOTE: It is recommended that each laboratory establishes and maintains its own reference ranges. The values given here are only to be used as a guideline.

Precision

see precision data of the following analyzers in "Appendix 1: Precision":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Method comparison

see method comparison data of the following analyzers in "Appendix 2: Method comparison":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Maintenance

ISE washing procedure for cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 and cobas pro ISE analytical unit.

The system maintenance procedures and frequencies stated in the operator's manual of the respective analyzer must be performed each day at the end of the daily sample run or after an elevated sample throughput.

cobas c 311:	The specially designated positions on the sample disk are used.
Position W1:	ISE Cleaning Solution
Position W2:	Activator

The ISE Wash procedure has to be manually selected out of maintenance items.

cobas c 501:	The specially labeled wash rack (green) is used.
Position 1:	Multiclean (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas 8000 ISE:	The specially labeled wash rack (green) is used.
Position 1:	Cell Cleaning Solution (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas c 303, cobas pro ISE:	The specially labeled wash rack (green) is used.
Position 1:	ISE Cleaning Solution (used for weekly wash rack)
Position 2:	ISE Cleaning Solution (used for daily wash rack)
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

The ISE systems require conditioning after cleaning and prior to calibration.

NOTE: Always use fresh solutions for cleaning.

ISE washing procedure for cobas ISE neo analytical unit

cobas ISE neo:	The ISE system wash tube holder is used.
Position CS:	ISE Cleaning Solution
Position A:	Activator

The maintenance task "ISE system wash" is scheduled and initiated automatically. For detailed description, refer to the operator's manual.

On-board stability of auxiliary reagents: ISE Cleaning Solution 4 days, Activator 4 days.

NOTE: Always exchange the tubes on the ISE tube holder, using new tubes for fresh reagents. **You must not refill them**, as this will lead to deterioration of the ISE measuring unit(s). Refer to the operator's manual for further information.

Appendix 1: Precision

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

	Repeatability			Intermediate precision		
Sample (on a **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	1.62	0.01	0.7	1.62	0.03	1.6
Plasma medium	4.97	0.04	0.7	4.97	0.04	0.8
Plasma high	9.46	0.06	0.6	9.46	0.07	0.7
Precinorm U	3.57	0.03	0.8	3.57	0.04	1.0
Precipath U	6.59	0.04	0.6	6.59	0.05	0.7
Urine low	5.12	0.03	0.6	5.12	0.04	0.7
Urine medium	52.08	0.32	0.6	52.08	0.67	1.3
Urine high	90.34	0.67	0.7	90.34	1.38	1.5
Liquichek 1	31.48	0.19	0.6	31.48	0.53	1.7
Liquichek 2	70.56	0.43	0.6	70.56	1.17	1.7

K Electrode

Potassium

cobas 8000 modular analyzer series: cobas 8000 ISE 900 / 1800 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

	Repeatability			Intermediate precision		
Sample (on a **cobas** 8000)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	2.03	0.01	0.5	2.03	0.03	1.6
Plasma medium	5.01	0.02	0.3	5.01	0.03	0.7
Plasma high	9.56	0.03	0.3	9.56	0.06	0.6
Precinorm U	3.60	0.02	0.4	3.60	0.03	0.9
Precipath U	6.61	0.02	0.3	6.61	0.04	0.5
Urine low	3.47	0.01	0.3	3.47	0.04	1.1
Urine medium	50.70	0.26	0.5	50.70	0.63	1.2
Urine high	93.48	0.58	0.6	93.48	1.82	1.9
Liquichek 1	30.64	0.20	0.6	30.64	0.32	1.0
Liquichek 2	66.22	0.61	0.9	66.22	1.14	1.7

cobas pure integrated solutions: cobas c 303 analytical unit

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

cobas pro integrated solutions: cobas pro ISE analytical unit

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 pro** ISE analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas pro** ISE analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	3.66	0.02	0.4	3.66	0.04	1.1
PCCC2[b)]	6.77	0.02	0.3	6.77	0.05	0.8
Human plasma 1	1.65	0.01	0.7	1.65	0.05	2.9
Human plasma 2	5.82	0.02	0.4	5.82	0.04	0.6
Human plasma 3	2.97	0.01	0.5	2.97	0.05	1.6
Human plasma 4	7.50	0.03	0.4	7.50	0.06	0.8
Human plasma 5	9.52	0.04	0.4	9.52	0.11	1.1
Human serum 1	1.59	0.01	0.7	1.59	0.04	2.3
Human serum 2	5.96	0.02	0.4	5.96	0.03	0.5
Human serum 3	2.96	0.01	0.4	2.96	0.04	1.2
Human serum 4	7.79	0.03	0.4	7.79	0.05	0.7
Human serum 5	9.86	0.05	0.5	9.86	0.08	0.8
Liquichek 1	31.1	0.24	0.8	31.1	0.55	1.8
Liquichek 2	69.9	0.45	0.6	69.9	1.56	2.2
Human urine 1	3.31	0.03	0.8	3.31	0.05	1.5
Human urine 2	50.8	0.30	0.6	50.8	1.01	2.0
Human urine 3	32.4	0.26	0.8	32.4	0.58	1.8
Human urine 4	82.4	0.85	1.0	82.4	2.07	2.5
Human urine 5	95.7	1.20	1.3	95.7	2.56	2.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

cobas pro integrated solutions: cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit

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 ISE neo** analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas** ISE neo analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	3.65	0.02	0.7	3.65	0.06	1.7
PCCC2[b)]	7.44	0.04	0.5	7.48	0.09	1.3
Human serum 1	1.76	0.01	0.6	1.76	0.06	3.2
Human serum 2	5.08	0.03	0.6	5.12	0.07	1.3
Human serum 3	3.64	0.02	0.5	3.64	0.04	1.2
Human serum 4	5.65	0.03	0.5	5.65	0.06	1.0
Human serum 5	9.70	0.05	0.5	9.76	0.08	0.8
Human plasma 1	1.69	0.01	0.7	1.69	0.06	3.5
Human plasma 2	5.03	0.03	0.7	5.06	0.06	1.1
Human plasma 3	3.50	0.02	0.6	3.52	0.05	1.4
Human plasma 4	5.56	0.03	0.5	5.56	0.06	1.0
Human plasma 5	9.70	0.05	0.5	9.77	0.09	0.9
Liquichek 1	30.3	0.21	0.7	30.3	0.60	2.0
Liquichek 2	71.7	0.67	0.9	71.7	2.00	2.8
Human urine 1	3.51	0.05	1.5	3.51	0.06	1.7
Human urine 2	48.5	0.39	0.8	48.5	1.22	2.5
Human urine 3	29.3	0.24	0.8	29.3	0.51	1.7
Human urine 4	75.2	0.61	0.8	75.2	2.09	2.8
Human urine 5	88.3	0.77	0.9	88.3	2.67	3.0

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Appendix 2: Method comparison

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

ISE values for human plasma and urine samples obtained on **cobas c** 501 analyzers (y) using ISE Standard High (compensated) as S3 Calibrator, were compared to those determined with the corresponding reference method (x) and with a **cobas c** 501 analyzer using ISE Compensator as S3 Calibrator.

The reference method used was: Flame Photometer IL 943 for potassium.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[16]	Coeff. (r)
x: flame photom. y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 106	1.59	9.56	y = 1.007x - 0.019	1.000

K Electrode

Potassium

Bias at 3.0 mmol/L = 0.002 (0.1 %) Bias at 5.8 mmol/L = 0.022 (0.4 %)					
x: **cobas c** 501 (S3 = ISE Compen-sator) y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 106	1.52	9.45	y = 1.006x + 0.024	1.000
Bias at 3.0 mmol/L = 0.042 (1.4 %) Bias at 5.8 mmol/L = 0.059 (1.0 %)					
x: flame photom. y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 105	4.00	97.2	y = 1.018x - 0.397	1.000
Bias at 20 mmol/L = 0.757 (3.8 %) Bias at 80 mmol/L = 1.837 (2.3 %)					
x: **cobas c** 501 (S3 = ISE Compen-sator) y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 105	4.05	97.4	y = 0.997x + 0.062	0.999
Bias at 20 mmol/L = 0.002 (0.0 %) Bias at 80 mmol/L = -0.178 (-0.2 %)					

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas 8000 modular analyzer series: cobas 8000 ISE 900 / 1800 module

ISE values for human plasma and urine samples obtained on a **cobas** 8000 analyzer (y) using ISE Standard High as S3 Calibrator, were compared with those determined using the corresponding reference method (x) and with **cobas c** 501 (x) using ISE Standard High as S3 Calibrator.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[16]	Coeff. (r)
x: flame photom. y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	1.54	10.57	y = 1x + 0.05	0.9994
Bias at 3.0 mmol/L = 0.050 (1.7 %) Bias at 5.8 mmol/L = 0.050 (0.9 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	1.59	10.59	y = 0.99x + 0.032	0.9999
Bias at 3.0 mmol/L = 0.002 (0.1 %) Bias at 5.8 mmol/L = -0.026 (-0.4 %)					
x: flame photom. y: **cobas** 8000 (S3 = ISE Standard High)	Urine / 101	3.1	99.5	y = 1.014x + 0.506	0.9997
Bias at 20 mmol/L = 0.786 (3.9 %) Bias at 80 mmol/L = 1.626 (2.0 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Urine / 101	2.97	102.04	y = 1.001x + 0.266	0.9998
Bias at 20 mmol/L = 0.286 (1.4 %) Bias at 80 mmol/L = 0.346 (0.4 %)					

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pure integrated solutions: cobas c 303 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas c** 303 ISE unit (y) were compared with a **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas c** 303 ISE unit (y) were compared with a **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[16]	Coeff. (r)
x: **cobas pro** ISE y: **cobas c** 303 ISE	Plasma / 120	1.52	9.95	y = 0.990x + 0.029	1.000
Bias at 3.5 mmol/L = -0.006 (-0.2 %) Bias at 5.5 mmol/L = -0.025 (-0.5 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Plasma / 120	1.55	10.0	y = 0.997x - 0.029	1.000
Bias at 3.5 mmol/L = -0.041 (-1.2 %) Bias at 5.5 mmol/L = -0.047 (-0.9 %)					

K Electrode

Potassium

x: **cobas pro** ISE y: **cobas c** 303 ISE	Serum / 116	1.62	9.81	y = 0.990x - 0.004	1.000
Bias at 3.5 mmol/L = -0.038 (-1.1 %) Bias at 5.5 mmol/L = -0.058 (-1.1 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Serum / 116	1.56	9.78	y = 0.984x + 0.059	1.000
Bias at 3.5 mmol/L = 0.002 (0.1 %) Bias at 5.5 mmol/L = -0.031 (-0.6 %)					
x: **cobas pro** ISE y: **cobas c** 303 ISE	Urine / 120	3.55	98.9	y = 0.983x + 0.290	1.000
x: **cobas c** 501 y: **cobas c** 303 ISE	Urine / 119	3.49	93.0	y = 0.950x + 0.628	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas pro ISE analytical unit

ISE values for human plasma samples obtained on a **cobas pro** ISE analytical unit (y) were compared with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas pro** ISE analytical unit (y) were compared with a **cobas c** 501 analyzer (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[16]	Coeff. (r)
x: **cobas c** 501 y: **cobas pro** ISE	Plasma / 120	1.71	9.57	y = 0.998x - 0.00004	1.000
Bias at 3.5 mmol/L = -0.041 (-1.2 %) Bias at 5.5 mmol/L = -0.064 (-1.2 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Serum / 120	1.62	10.0	y = 1.004x - 0.082	1.000
Bias at 3.5 mmol/L = -0.068 (-1.9 %) Bias at 5.5 mmol/L = -0.060 (-1.1 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Urine / 119	3.28	96.4	y = 1.035x - 0.507	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas** ISE neo analytical unit (y) were compared with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

ISE values for human urine samples obtained on a **cobas** ISE neo analytical unit (y) were compared with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[16]	Coeff. (r)
x: **cobas c** 501 y: **cobas** ISE neo	Serum / 118	1.62	9.93	y = 1.000x - 0.0400	1.000
Bias at 3.5 mmol/L = -0.0400 (-1.1 %) Bias at 5.5 mmol/L = -0.0400 (-0.7 %)					
x: **cobas pro** ISE y: **cobas** ISE neo	Serum / 119	1.52	9.89	y = 1.000x - 0.0200	1.000
Bias at 3.5 mmol/L = -0.0200 (-0.6 %) Bias at 5.5 mmol/L = -0.0200 (-0.4 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Plasma / 116	1.64	9.69	y = 1.000x - 0.0400	0.999
Bias at 3.5 mmol/L = -0.0400 (-1.1 %) Bias at 5.5 mmol/L = -0.0400 (-0.7 %)					
x: **cobas pro** ISE y: **cobas** ISE neo	Plasma / 115	1.62	9.72	y = 1.008x - 0.0378	1.000
Bias at 3.5 mmol/L = -0.00840 (-0.2 %) Bias at 5.5 mmol/L = 0.00840 (0.2 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Urine / 113	3.41	93.4	y = 1.056x - 0.777	1.000
x: **cobas pro** ISE y: **cobas** ISE neo	Urine / 113	3.43	94.3	y = 1.035x - 0.489	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

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K Electrode

Potassium

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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):

Cont.	Quantity contained in the package
CONTENT	Quantity contained in the package
GTIN	Global Trade Item Number
INSTALL BEFORE	Latest date by which the electrode has to be installed on the analyzer
RoHS	Directive for the restriction of the use of certain hazardous substances in electrical and electronic equipment

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

CE 0123

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LDHI2

cobas®

Lactate Dehydrogenase acc. to IFCC ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057958190*	08057958500	Lactate Dehydrogenase acc. to IFCC ver.2 (850 tests)	System-ID 2081 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057958214*	08057958500	Lactate Dehydrogenase acc. to IFCC ver.2 (850 tests)	System-ID 2081 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

LDHI2: ACN 20810

LDHI2P: ACN 20811 (with automatic sample pre-dilution)

Intended use

In vitro test for the quantitative determination of lactate dehydrogenase in human serum and plasma on **cobas c** systems.

Summary

Lactate dehydrogenase (LDH) measurements, performed with this assay in human serum and plasma are used as an aid for diagnosis and monitoring of various clinical conditions associated with tissue damage (e.g. myocardial infarction, liver disorders such as severe toxic liver injury, malignant tumors such as leukemias), and for the prognosis of certain solid tumors.

LDH is a nicotinamide dinucleotide (NAD^+)-dependent oxidoreductase and catalyzes the reversible transformation of lactate to pyruvate under anaerobic conditions, coupled with the oxidation of NADH to NAD^+.[1,2] LDH is widely distributed in tissue, particularly in the heart, liver, muscles and kidneys. Upon cell injury and/or necrosis, LDH is released into the circulation. LDH in serum can be separated into five different isoenzymes based on their electrophoretic mobility. Each isoenzyme is a tetramer composed of two different subunits. These two subunits have been designated heart and muscle, based on their polypeptide chains. There are two homotetramers, LDH-1 (heart) and LDH-5 (muscle), and three hybrid isoenzymes.[1,2] In disease conditions, the LDH activity measured in serum is dependent on the isoenzymes entering the plasma from the tissues, the elimination rate of the isoenzymes and their subunits.[1,2]

Elevated serum levels of LDH have been observed in a variety of disease states.[1] The highest levels are seen in patients with megaloblastic anemia (up to 50 times the upper reference limit), disseminated carcinoma, sepsis and other causes of shock (because of damage to multiple organs).[1,2] Moderate increases occur in muscular disorders, nephrotic syndrome and cirrhosis. Mild increases in LDH activity have been reported in cases of myocardial or pulmonary infarction, leukemia, hemolytic anemia and non-viral hepatitis. Because of its wide tissue distribution and its lack of tissue specificity for diagnostic use, serum LDH measurement is relevant in broad indications like hematology and oncology.[1,3] LDH is routinely used as a marker of hemolysis in sickle cell disease, along with the elevated reticulocyte count, elevated levels of unconjugated bilirubin concentration and aspartate aminotransferase, and decreased level of serum haptoglobin. While none of those parameters are specific markers of hemolysis, LDH has however been considered the most relevant biomarker of hemolysis and has been proposed as a diagnostic and prognostic marker of acute and chronic complications of sickle cell disease.[4] LDH has demonstrated to have prognostic significance in several tumor types, including pancreatic cancer, lung cancer, advanced thymic carcinoma, osteosarcoma, renal cell carcinoma, colorectal cancer, melanoma, prostate cancer, bladder cancer, and urologic cancer.[1,5,6,7,8,9] As a biochemical marker of tumor burden, LDH has been incorporated into several prognostic scores and staging for several types of cancer (e.g. renal cell carcinoma, melanoma and colorectal cancer).[5,6]

The method described here is derived from the formulation recommended by the IFCC[10,11] and was optimized for performance and stability.

Test principle

UV assay

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.

$$\text{L-Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometrically measuring the increase in absorbance.

Reagents - working solutions

R1 N-methylglucamine: 400 mmol/L, pH 9.4 (37 °C); lithium lactate: 62 mmol/L; stabilizers

R3 NAD: 62 mmol/L; stabilizers; preservatives

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Hazardous components:

- hydroxylammonium chloride
- 2-methyl-2H-isothiazol-3-one hydrochloride

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

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
Plasma: Li-heparin plasma. Plasma must be free from cells.

Caution: Plasma from primary tubes handled according to the manufacturer's instructions can still contain cells, leading to implausibly high results. One option for these cases is an application with automatic sample pre-dilution (ACN 20811). Alternatively it is recommended to transfer the plasma from the primary tube to a secondary sample tube.

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.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

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

Stability:[12]	7 days at 15-25 °C The sample may be stored for 4 days at 2-8 °C or 6 weeks at -20 °C (±5 °C). In connection with certain diseases (e.g. hepatopathy, diseases of skeletal muscle, malignant tumors), the LDH-4 and LDH-5 isoenzyme portions are increased and unstable in cooled and frozen samples; this may lead to an incorrect LDH value in samples collected from patients suffering from such diseases.

Freeze only once.

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	79 µL	–	
R3	16 µL	–	
Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.2 µL	–	–
Decreased	2.8 µL	25.0 µL	56 µL
Increased	2.2 µL	–	–
Sample volumes LDHI2P	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	11.0 µL	16.0 µL	64 µL
Decreased	4.4 µL	16.0 µL	64 µL
Increased	11.0 µL	16.0 µL	64 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: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - 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 the original IFCC[11] formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

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 26 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 20 U/L of initial values of samples ≤ 200 U/L and within ± 10 % for samples > 200 U/L

Icterus:[13] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

LDHI2

Lactate Dehydrogenase acc. to IFCC ver.2

Hemolysis:[13] No significant interference up to an H index of 15 (approximate hemoglobin concentration: 9.6 µmol/L or 15 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[13] No significant interference up to an L index of 900. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

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

10-1000 U/L (0.17-16.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 U/L (0.17 µkat/L)
Limit of Detection	= 10 U/L (0.17 µkat/L)
Limit of Quantitation	= 10 U/L (0.17 µkat/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 activity 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 activity samples.

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

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

Expected values

U/L

Acc. to IFCC measured at 37 °C:[17]

Females	135-214 U/L
Males	135-225 U/L
Children (2-15 y)	120-300 U/L
Newborns (4-20 d)	225-600 U/L

Consensus values:[18]

Males & Females	up to 250 U/L

µkat/L

Acc. to IFCC measured at 37 °C:[17]

Females	2.25-3.55 µkat/L
Males	2.25-3.75 µkat/L
Children (2-15 y)	2.00-5.00 µkat/L
Newborns (4-20 d)	3.75-10.0 µkat/L

Consensus values:[18]

Males & Females	up to 4.2 µkat/L

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.

LDHI2

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	172	1.06	0.6
PCCC2[b)]	294	1.35	0.5
Human serum 1	22.4	0.646	2.9
Human serum 2	164	1.29	0.8
Human serum 3	265	1.56	0.6
Human serum 4	520	2.09	0.4
Human serum 5	943	3.31	0.4
Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	166	1.43	0.9
PCCC2[b)]	287	2.20	0.8
Human serum 1	22.4	0.779	3.5
Human serum 2	164	2.38	1.4
Human serum 3	265	2.32	0.9
Human serum 4	520	4.30	0.8
Human serum 5	943	5.65	0.6

LDHI2P

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	165	1.01	0.6
PCCC2[b)]	292	1.26	0.4
Human serum 1	21.5	0.555	2.6
Human serum 2	164	1.47	0.9
Human serum 3	262	1.78	0.7
Human serum 4	519	1.80	0.3
Human serum 5	941	2.92	0.3

LDHI2

Lactate Dehydrogenase acc. to IFCC ver.2

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	168	1.25	0.7
PCCC2[b)]	292	2.08	0.7
Human serum 1	21.5	0.783	3.6
Human serum 2	164	2.48	1.5
Human serum 3	262	2.39	0.9
Human serum 4	520	4.43	0.9
Human serum 5	940	5.58	0.6

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

Lactate dehydrogenase 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).

LDHI2

Sample size (n) = 66

Passing/Bablok[19]	Linear regression
y = 0.999x - 2.72 U/L	y = 1.001x - 3.32 U/L
τ = 0.992	r = 1.000

The sample activities were between 19.8 and 973 U/L.

LDHI2P

Sample size (n) = 66

Passing/Bablok[19]	Linear regression
y = 0.997x - 2.26 U/L	y = 1.003x - 3.70 U/L
τ = 0.982	r = 1.000

The sample activities were between 19.8 and 973 U/L.

Lactate dehydrogenase 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).

LDHI2

Sample size (n) = 60

Passing/Bablok[19]	Linear regression
y = 1.007x - 0.451 U/L	y = 1.016x - 3.51 U/L
τ = 0.982	r = 1.000

The sample activities were between 60.1 and 960 U/L.

LDHI2P

Sample size (n) = 60

Passing/Bablok[19]	Linear regression
y = 0.998x - 0.521 U/L	y = 0.999x - 1.75 U/L
τ = 0.983	r = 1.000

The sample activities were between 62.1 and 973 U/L.

Lactate dehydrogenase 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).

LDHI2

Sample size (n) = 65

Passing/Bablok[19]	Linear regression
y = 1.002x + 0.668 U/L	y = 1.008x - 0.274 U/L
τ = 0.963	r = 1.000

The sample concentrations were between 14.1 and 955 U/L.

LDHI2P

Sample size (n) = 65

Passing/Bablok[19]	Linear regression
y = 1.002x + 1.55 U/L	y = 1.001x + 1.91 U/L
τ = 0.974	r = 1.000

The sample concentrations were between 13.1 and 952 U/L.

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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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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LDLC3

cobas®

LDL-Cholesterol Gen.3

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057966190*	08057966500	LDL-Cholesterol Gen.3 (600 tests)	System-ID 2082 002	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057966214*	08057966500	LDL-Cholesterol Gen.3 (600 tests)	System-ID 2082 002	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

12172623122	Calibrator f.a.s. Lipids (3 × 1 mL)	Code 20424	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

LDLC3: ACN 20820

Intended use

In vitro test for the quantitative determination of LDL-cholesterol in human serum and plasma on **cobas c** systems.

Summary

Measurements of LDL-cholesterol, performed with this assay in human serum or plasma, are used for screening, aid in diagnosis and monitoring of dyslipidaemias as well as for assessment of cardiovascular risk such as in ASCVD and CHD.

The Low Density Lipoproteins (LDLs) are derived from VLDLs (Very Low Density Lipoproteins) that are enriched in triglycerides by the action of various lipolytic enzymes and are synthesized in the liver. The elimination of LDL from plasma takes place mainly by liver parenchymal cells via specific LDL receptors. Elevated LDL concentrations in blood and an increase in their residence time (coupled with an increase in the biological modification rate) result in the destruction of the endothelial function and a higher LDL-cholesterol uptake in the monocyte/macrophage system as well as into smooth muscle cells in vessel walls. The majority of cholesterol stored in atherosclerotic plaques originates from LDL.

LDL particles play a key role in causing and influencing the progression of atherosclerotic cardiovascular diseases (ASCVDs), including coronary heart disease (CHD), ischemic stroke, peripheral artery disease, and aortic aneurysm.[1] Atherosclerosis is a condition when the arteries are hardened and narrowed by plaque formation, which is a deposition of fats, cholesterol and other substances in and on the artery walls. Over time, the plaques can remain asymptomatic or become obstructive (stable angina). Eventually, plaque rupture can occur, where the contact of blood with the exposed plaque content can lead to thrombus formation and subsequent myocardial infarction or stroke.[1] The LDL-cholesterol value is a measure of the cholesterol mass carried by LDL particles and is used as a clinical predictor for ASCVD.[2] As a result, measurements of LDL-cholesterol are used for screening, aid in diagnosis and monitoring of dyslipidaemias, as well as for assessment of cardiovascular risk such as in ASCVD and CHD.[3,4] Therapies focusing on lipid reduction primarily target the reduction of LDL-cholesterol which is then expressed in an improvement of the endothelial function, prevention of atherosclerosis and reducing its progression as well as preventing plaque rupture and myocardial infarction.[3,4] Non-fasting sample results are slightly lower than fasting results.[5]

Various methods are available for the determination of LDL-cholesterol such as ultracentrifugation as the reference method, lipoprotein electrophoresis, high performance liquid chromatography (HPLC) and precipitation methods.[6,7] In the precipitation methods apolipoprotein B-containing LDL-cholesterol is, for example, precipitated using either polyvinyl sulfate, dextran sulfate or polycyclic anions. The LDL-cholesterol content is usually calculated from the difference between total cholesterol and cholesterol in the remainder (VLDL and HDL-cholesterol) in the supernate after precipitation with polyvinyl sulfate and dextran sulfate.[8] Lipid Research Clinics recommend a combination of ultracentrifugation and precipitation methods using polyanions in the presence of divalent cations. The precipitation methods are, however, time-consuming, cannot be automated and are susceptible to interference by hyperlipidemic serum, particularly at high concentrations of free fatty acids. A more recent method is based on the determination of LDL-cholesterol after the sample is subjected to immunoadsorption and centrifugation.[9]

The calculation of the LDL-cholesterol concentration according to Friedewald's formula is based on 2 cholesterol determinations (total cholesterol and HDL-cholesterol) and 1 triglyceride determination.[10]

Friedewald's formula for calculation of LDL-cholesterol presumes that a direct relationship exists between VLDL-cholesterol and triglycerides in fasting blood samples (VLDL-cholesterol = Trigl./5 mg/dL, VLDL-cholesterol = Trigl./2.2 mmol/L). The bias in calculating LDL-cholesterol using this assumption is only acceptable in samples with a triglyceride concentration < 2.0 mmol/L (177 mg/dL).[11,12] Even in the presence of small amounts of chylomicrons or abnormal lipoproteins, the formula gives rise to artificially low LDL-cholesterol values. Non-fasting samples cannot be used for the calculation of LDL-cholesterol because they contain a high concentration of chylomicrons and in many cases the limit of acceptable triglyceride concentration is exceeded. For these reasons, a simple and reliable method for routine measurement of LDL-cholesterol without any preparatory steps was developed. This automated method for the direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase - cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL < LDL.

The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum and plasma samples.

Comparable non-fasting results were observed with the beta quantification method.[13] This direct assay meets the 1995 NCEP goals of < 4 % total coefficient of variation (CV), bias ≤ 4 % versus reference method, and ≤ 12 % total analytical error.[14,15,16]

Test principle

Homogeneous enzymatic colorimetric assay

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{cholesterol esterase}]{\text{detergent}} \text{cholesterol} + \text{free fatty acids (selective micellary solubilization)}$$

LDLC3

LDL-Cholesterol Gen.3

cobas®

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{LDL-cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \Delta^4\text{-cholestenone} + H_2O_2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$$2\ H_2O_2 + \text{4-aminoantipyrine} + \text{EMSE}^{a)} + H_2O + H^+ \xrightarrow{\text{peroxidase}} \text{red purple pigment} + 5\ H_2O$$

a) N-ethyl-N-(3-methylphenyl)-N-succinylethylenediamine

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and EMSE to form a red purple dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

Reagents - working solutions

R1 Bis-tris[b)] buffer: 20.1 mmol/L, pH 7.0; 4-aminoantipyrine: 0.98 mmol/L; ascorbate oxidase (AOD, Acremonium spec.): ≥ 66.7 µkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 166.7 µkat/L; BSA: 4.0 g/L; preservative

R3 MOPS[c)] buffer: 20.1 mmol/L, pH 7.0; EMSE: 2.16 mmol/L; cholesterol esterase (Pseudomonas spec.): ≥ 33.3 µkat/L; cholesterol oxidase (recombinant from E. coli): ≥ 31.7 µkat/L; peroxidase (recombinant from Basidiomycetes): ≥ 333.3 µkat/L; BSA: 4.0 g/L; detergents; preservative

b) bis(2-hydroxyethyl)-amino-tris-(hydroxymethyl)-methane

c) 3-morpholinopropane-1-sulfonic acid

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H411	Toxic to aquatic life with long lasting effects.

Prevention:

P261	Avoid breathing mist or vapours.
P273	Avoid release to the environment.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P391	Collect spillage.

Hazardous components:

- reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

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
Plasma: Li-heparin, K_2- and K_3-EDTA plasma.

Fasting and non-fasting samples can be used.[9]

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.

Stability:[17,18]	7 days at 2-8 °C
	12 months at -20 °C (± 5 °C)
	12 months at -70 °C (± 5 °C)

Freeze only once.

It is reported that EDTA stabilizes lipoproteins.[16]

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)	700/600 nm	
Reagent pipetting		Diluent (H_2O)
R1	82 µL	–
R3	27 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*

Normal	1.1 µL	–	–
Decreased	5.5 µL	10 µL	90 µL
Increased	1.1 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: H_2O
	S2: C.f.a.s. Lipids
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 the beta quantification method as defined in the recommendations in the LDL Cholesterol Method Certification Protocol for Manufacturers.[19]

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 26 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 in the unit mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L × 38.66 = mg/dL
	mmol/L × 0.3866 = g/L

Limitations – interference

Criterion: Recovery within ± 0.40 mmol/L of initial values of samples ≤ 4.0 mmol/L and within ±10 % for samples > 4.0 mmol/L.

Icterus:[20] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[20] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[20] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

No significant interference from HDL-C (≤ 3.03 mmol/L or ≤ 117 mg/dL), VLDL-C (≤ 3.63 mmol/L or ≤ 140 mg/dL), or chylomicrons (≤ 22.6 mmol/L or ≤ 2000 mg/dL triglycerides).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[21,22]

Nicotinic acid (Niacin), statins (Simvastatin) and fibrates (Clofibrate) tested at therapeutic concentration ranges did not interfere.

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-acetylcysteine at the therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low LDL-C results. Venipuncture should be performed prior to the administration of metamizole. Venipuncture immediately after or during the administration of metamizole may lead to falsely low results.

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 28.4 mmol/L (500 mg/dL).

Abnormal liver function affects lipid metabolism; consequently HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the LDL-cholesterol result is significantly negatively biased versus beta quantification results.

EDTA plasma may cause decreased values compared to serum.[23]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[24]

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

0.10-14.2 mmol/L (3.87-549 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.10 mmol/L (3.87 mg/dL)
Limit of Detection	= 0.10 mmol/L (3.87 mg/dL)
Limit of Quantitation	= 0.10 mmol/L (3.87 mg/dL)

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 for LDL-C is 0.10 mmol/L determined in accordance with the guidelines in CLSI document EP17-A2, based on a minimum of 48 determinations, and a total error goal of 10 % calculated using RMS error model.

Expected values[25]

Levels in terms of risk for coronary heart disease.

mmol/L*

Adult levels:

Optimal	< 2.59 mmol/L
Near optimal/above optimal	2.59-3.34 mmol/L
Borderline high	3.37-4.12 mmol/L
High	4.14-4.89 mmol/L
Very high	≥ 4.92 mmol/L

* calculated by unit conversion factor

mg/dL

Adult levels:

Optimal	< 100 mg/dL
Near optimal/above optimal	100-129 mg/dL

Borderline high	130-159 mg/dL
High	160-189 mg/dL
Very high	≥ 190 mg/dL

Risk classification of patients and treatment therapies are described in international guidelines.[26]

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.

Repeatability	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
PCCC1[d)]	1.43	0.00779	0.5
PCCC2[e)]	2.60	0.0129	0.5
Human serum 1	0.163	0.00288	1.8
Human serum 2	1.27	0.00627	0.5
Human serum 3	2.57	0.0131	0.5
Human serum 4	7.54	0.0358	0.5
Human serum 5	13.8	0.0627	0.5

Intermediate precision	*Mean* *mmol/L*	*SD* *mmol/L*	*CV* *%*
PCCC1[d)]	1.43	0.0147	1.0
PCCC2[e)]	2.60	0.0298	1.1
Human serum 1	0.163	0.00337	2.1
Human serum 2	1.27	0.00940	0.7
Human serum 3	2.57	0.0160	0.6
Human serum 4	7.54	0.0509	0.7
Human serum 5	13.8	0.0931	0.7

d) PreciControl ClinChem Multi 1

e) PreciControl ClinChem Multi 2

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

LDL-cholesterol values for human serum 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) = 86

Passing/Bablok[27]	Linear regression
y = 1.021x + 0.0546 mmol/L	y = 1.015x + 0.0745 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.120 and 14.0 mmol/L.

LDL-cholesterol values for human serum 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) = 86

Passing/Bablok[27]	Linear regression
y = 1.005x + 0.0713 mmol/L	y = 1.008x + 0.0574 mmol/L
τ = 0.981	r = 1.000

The sample concentrations were between 0.130 and 13.8 mmol/L.

LDL-cholesterol values for human serum 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) = 75

Passing/Bablok[27]	Linear regression
y = 0.973x - 0.0361 mmol/L	y = 0.981x - 0.0623 mmol/L
τ = 0.986	r = 1.000

The sample concentrations were between 0.208 and 13.9 mmol/L.

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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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

LIPC

cobas®

Lipase colorimetric assay

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057982190	08057982500	Lipase colorimetric assay (200 tests)	System-ID 2085 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

LIP: ACN 20850

Intended use

Enzymatic in vitro test for the quantitative determination of lipase in human serum and plasma on **cobas c** systems.

Summary

Lipase measurements, performed with this assay in human serum and plasma, are used as an aid in the diagnosis and monitoring of various pancreatic conditions, particularly acute pancreatitis.

Lipases are triglyceride hydrolases which catalyze the cleavage of triglycerides into fatty acids and glycerol.[1,2] Most of the lipase activity found in serum derives from pancreatic acinar cells, but some is secreted by gastric and intestinal mucosa.[1,2] Human pancreatic lipase is a glycoprotein with a molecular weight of 45-48 kDa.[1,2,3] It is secreted into the duodenum through the duct system of the pancreas, and the concentration in blood is normally very low: the concentration gradient between pancreatic tissue and serum lipase is approximately 20,000-fold. Upon pancreatic injury, the pancreas starts to release the lipase into blood at higher amounts. This can occur in conditions such as acute pancreatitis, chronic pancreatitis, pancreatic cancer, or pancreatic duct obstruction. Therefore, the measurement of pancreatic lipase in blood can be used as an aid to diagnose acute pancreatitis and other pancreatic diseases.[2,3]

In addition to α-amylase, pancreatic lipases have for many years been undeniably the most important clinical chemistry parameters for the differential diagnosis of diseases of the pancreas.[4,5,6,7] The lipase activity determination has gained increasing international recognition because of its high specificity and rapid response. After acute pancreatitis the lipase activity increases within 4-8 hours, reaches a peak after 24 hours and decreases after 8 to 14 days.[2,4,5,6]

Lipase activity in serum can also be influenced by factors other than pancreatic disorders, such as kidney disease, intestinal ischemia, or certain medications.[1,2] Therefore, clinical interpretation of lipase levels should be done in conjunction with a comprehensive assessment of the patient's medical history, symptoms, and other diagnostic tests.

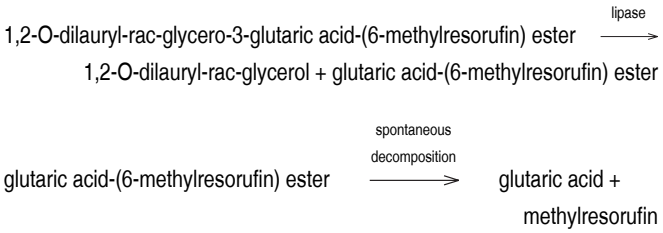
The hydrolyzation action of lipase can only take place when the substrate is present in an emulsified form and the rate of action depends on the free surface area of the substrate. Bile and co-lipase are thus essential for the activity of pancreatic lipase as bile helps emulsify fats, increasing their surface area for lipase action, and co-lipase enhances the binding and activity of lipase at the lipid-water interface.[1]

Numerous methods have been described for the determination of lipase which determine the decrease in substrate turbidimetrically or nephelometrically or determine degradation products.[1,3,8,9] The method of this assay is based on the cleavage of a specific chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester emulsified with bile acids. The pancreatic enzyme activity is determined specifically by the combination of bile acid and colipase used in this assay. Virtually no lipase activity is detected in the absence of colipase. Colipase only activates pancreatic lipase, but not other lipolytic enzymes found in serum. The high amount of cholates ensures that the esterases present in the serum do not react with the chromogenic substrate due to the highly negative surface charge.

Test principle[10,11,12,13]

Enzymatic colorimetric assay with 1,2-O-dilauryl-rac-glycero-3-glutaric-acid-(6-methylresorufin) ester as substrate.

The chromogenic lipase substrate 1,2-O-dilauryl-rac-glycero-3-glutaric-acid-(6-methylresorufin) ester is cleaved by the catalytic action of alkaline lipase solution to form 1,2-O-dilauryl-rac-glycerol and an unstable intermediate, glutaric acid-(6-methylresorufin) ester. This decomposes spontaneously in alkaline solution to form glutaric acid and methylresorufin. Addition of detergent and colipase increases the specificity of the assay for pancreatic lipase.



The color intensity of the red dye formed is directly proportional to the lipase activity and can be determined photometrically.

Reagents - working solutions

R1 BICIN[a)] buffer: 50 mmol/L, pH 8.0; colipase (porcine pancreas): ≥ 0.9 mg/L; Na-deoxycholate: 1.6 mmol/L; calcium chloride: 10 mmol/L; detergent; preservative

R3 Tartrate buffer: 10 mmol/L, pH 4.16; 1,2-O-dilauryl-rac-glycero-3-glutaric acid-(6-methylresorufin) ester: 0.27 mmol/L; taurodeoxycholate: 8.8 mmol/L; detergent; preservative

a) BICIN = N,N-bis(2-hydroxyethyl)glycine

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

LIPC

cobas®

Lipase colorimetric assay

H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.

Prevention:

P261	Avoid breathing mist or vapours.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:	4 weeks

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
Plasma: Li-heparin plasma

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.

Stability in serum:[14]	7 days at 20-25 °C
	7 days at 4-8 °C
	1 year at -20 °C (±5 °C)

Freeze only once.

Stability in plasma:	1 week at 15-25 °C
	1 week at 2-8 °C
	2 months at -20 °C (±5 °C)

Freeze only once.

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)	700/570 nm		
Reagent pipetting		Diluent (H_2O)	
R1	60 µL	15 µL	
R3	36 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	10	90
Increased	1.5 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: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot 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 manually against Roche reagent using the substrate-specific absorptivity, ε.

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 4 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 6 U/L of initial values of samples ≤ 60 U/L and within ± 10 % for samples > 60 U/L.

Icterus:[15] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[15] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62 µmol/L or 100 mg/dL).

Lipemia (Intralipid):[15] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

LIPC

Lipase colorimetric assay

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

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

3-300 U/L (0.05-5.01 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/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 activity 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 activity samples.

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

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

Expected values[19]

Adults: 13-60 U/L (0.22-1.00 µkat/L*)

*calculated by unit conversion factor

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.

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[b)]	45.5	0.295	0.6
PCCC2[c)]	102	0.425	0.4
Human serum 1	6.59	0.230	3.5
Human serum 2	40.2	0.245	0.6
Human serum 3	94.4	0.445	0.5
Human serum 4	152	0.617	0.4
Human serum 5	250	0.866	0.3

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[b)]	45.5	0.498	1.1
PCCC2[c)]	99.3	1.08	1.1
Human serum 1	6.59	0.267	4.1
Human serum 2	40.2	0.368	0.9
Human serum 3	94.4	1.01	1.1
Human serum 4	142	1.57	1.1
Human serum 5	250	2.79	1.1

b) PreciControl ClinChem Multi 1

c) PreciControl ClinChem Multi 2

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

Lipase 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) = 72

Passing/Bablok[20]	Linear regression
y = 1.013x + 0.718 U/L	y = 1.033x + 0.415 U/L
τ = 0.962	r = 0.998

The sample activities were between 3.29 and 261 U/L.

Lipase 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) = 71

Passing/Bablok[20]	Linear regression
y = 1.039x + 0.475 U/L	y = 1.027x + 0.689 U/L
τ = 0.975	r = 0.999

The sample activities were between 4.30 and 282 U/L.

Lipase 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) = 75

Passing/Bablok[20]	Linear regression
y = 0.980x + 0.265 U/L	y = 0.976x + 0.625 U/L
τ = 0.955	r = 1.000

The sample concentrations were between 5.06 and 288 U/L.

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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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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MG2

cobas®

Magnesium Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058016190*	08058016500	Magnesium Gen.2 (690 tests)	System-ID 2089 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058016214*	08058016500	Magnesium Gen.2 (690 tests)	System-ID 2089 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

MG2: ACN 20890 (Serum/plasma)

MG2U: ACN 20891 (Urine)

Intended use

In vitro test for the quantitative determination of magnesium in human serum, plasma and urine on **cobas c** systems.

Summary

Magnesium measurements, performed with this assay, in human serum, plasma and urine are used as an aid in diagnosis and monitoring disorders of magnesium metabolism associated with hypomagnesemia (magnesium deficiency) and hypermagnesemia (magnesium excess).

Magnesium is mainly found in the intracellular space (40 %) and in bones and teeth (60 %). Approximately 0.3 % of the body's total magnesium is found in serum.[1] As important intracellular cation, Mg^{2+} is a cofactor in more than 300 enzyme-catalyzed reactions involved in phosphorylation, protein synthesis, and DNA metabolism processes. All ATP-dependent enzymatic reactions require Mg^{2+} as a cofactor. In addition, magnesium is a dynamic ion for transcellular transport, altering membrane potentials and ion transport. It is involved in neuromuscular conduction and excitability of skeletal and cardiac muscle.[2]

Approximately 99 % of magnesium ions are stored in bone, skeletal muscle and other soft tissues and less than 1 % is present in the extracellular fluid. The Mg^{2+} serum level is kept constant within very narrow limits (0.7-1.10 mmol/L). Approximately 20 % of this is protein bound (especially to albumin), 65 % is ionized and the rest is complexed with various anions such as phosphate and citrate.[3] Serum levels are mainly regulated via the kidneys, especially via the ascending loop of Henle.[4,5] Emerging evidence suggests that the serum magnesium/calcium quotient is an important indicator of magnesium status and/or turnover.[1]

Hypomagnesemia is common, with a prevalence of up to 15 % in the general population and up to 65 % in patients in the intensive care units.[5] Hypomagnesemia is usually due to loss or impaired absorption of magnesium from the gastrointestinal tract or increased excretion by the kidneys.[2,5] Symptomatic magnesium depletion is often correlated with multiple other biochemical abnormalities, such as hypokalaemia, hypocalcaemia and metabolic acidosis. Manifestations of severe hypomagnesaemia include neuromuscular symptoms (muscular weakness, apathy, tremors, paraesthesia, tetany, vertical nystagmus and positive Chvostek and Trousseau signs) and cardiovascular manifestations (e.g. atrial and ventricular arrhythmias).[4] Intravenous magnesium is usually prescribed in cases of symptomatic hypomagnesaemia, while oral replacement is indicated for asymptomatic patients.[4]

Hypermagnesemia is generally occurring in the setting of renal insufficiency (acute and chronic renal failure) and excessive magnesium intake resulting in neuromuscular and cardiovascular manifestations as well as non-specific manifestations like nausea, vomiting and cutaneous flushing.[4]

In addition to atomic absorption spectrometry (AAS), complexometric methods can also be used to determine magnesium.[2,6]

The method described here is based on the reaction of magnesium with xylidyl blue in alkaline solution containing EGTA to mask the calcium in the sample.[7]

Urine magnesium is also often measured as part of a magnesium loading test.[8]

Test principle[7]

Colorimetric endpoint method

- Sample and addition of R1
- Addition of R2 and start of reaction:

  In alkaline solution, magnesium forms a purple complex with xylidyl blue, diazonium salt. The magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance.

Reagents - working solutions

R1 TRIS[a)]/6-aminocaproic acid buffer: 500 mmol/L, pH 11.25; EGTA: 129 µmol/L; preservative

R3 Xylidyl blue: 0.28 mmol/L; detergent; preservative

a) TRIS = Tris(hydroxymethyl)-aminomethane

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H315 Causes skin irritation.

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

P302 + P352 IF ON SKIN: Wash with plenty of water.

P332 + P313 If skin irritation occurs: Get medical advice/attention.

P337 + P313 If eye irritation persists: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 weeks

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
Plasma: Li-heparin plasma

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.

Chelating anticoagulants such as EDTA, fluoride and oxalate must be avoided.

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.

Stability in *serum/plasma*:[9]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

Urine:

Urine samples should be acidified to pH 1 with concentrated HCl to prevent precipitation of magnesium ammonium phosphate. Collect urine samples in metal-free container.[10] Urine samples are automatically prediluted with 0.9 % NaCl by the instrument. If stabilizers are added to the sample, the sample index feature must not be used.

Stability in *urine*:[9]	3 days at 15-25 °C
	3 days at 2-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

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)	505/600 nm	
Reagent pipetting		Diluent (H_2O)
R1	78 µL	–
R3	78 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.4 µL	–	–
Decreased	1.2 µL	–	–
Increased	2.4 µL	–	–

Application for urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	505/600 nm	
Reagent pipetting		Diluent (H_2O)
R1	78 µL	-
R3	78 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.4 µL	20 µL	90 µL
Decreased	2.4 µL	10 µL	100 µL
Increased	2.4 µL	20 µL	90 µL

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

Calibration

Application for serum/plasma (ACN 20890)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - every 4 weeks on-board - as required following quality control procedures

Application for urine (ACN 20891)
Transfer of calibration from serum/plasma application (ACN 20890)

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

Traceability: This method has been standardized against atomic absorption spectrometry.

Quality control

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

MG2

Magnesium Gen.2

cobas®

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 26 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 in the unit mmol/L (mg/dL, mg/L, mval/L).

Conversion factors:	mmol/L x 2.43 = mg/dL
	mmol/L x 24.3 = mg/L
	mmol/L x 2.0 = mval/L
	mval/L = mEq/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a magnesium concentration of 0.7 mmol/L (1.7 mg/dL, 1.4 mval/L).

Serum/plasma

Icterus:[11] No significant interference up to an I index of 60 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 60 mg/dL or 1026 µmol/L).

Hemolysis:[11] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 496 µmol/L (800 mg/dL)).

Hemolysis elevates results depending on the content of the analyte in the lysed erythrocytes.

Lipemia (Intralipid):[11] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[14]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13]

Criterion: Recovery within ± 10 % of initial value at a magnesium concentration of 1.7 mmol/L (4.1 mg/dL, 3.4 mval/L).

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

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

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

Serum/plasma

0.10-2.0 mmol/L (0.243-4.86 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Urine

0.56-11.0 mmol/L (1.36-26.7 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma

Limit of Blank	= 0.05 mmol/L (0.122 mg/dL)
Limit of Detection	= 0.10 mmol/L (0.243 mg/dL)
Limit of Quantitation	= 0.10 mmol/L (0.243 mg/dL)

Urine

Limit of Blank	= 0.28 mmol/L (0.68 mg/dL)
Limit of Detection	= 0.56 mmol/L (1.36 mg/dL)
Limit of Quantitation	= 0.56 mmol/L (1.36 mg/dL)

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 magnesium samples.

Expected values[15]

mmol/L

Serum/plasma:

Newborn:	0.62-0.91 mmol/L
5 months-6 years:	0.70-0.95 mmol/L
6-12 years:	0.70-0.86 mmol/L
12-20 years:	0.70-0.91 mmol/L
Adults:	0.66-1.07 mmol/L
60-90 years:	0.66-0.99 mmol/L
> 90 years:	0.70-0.95 mmol/L
Urine (24 h):	3.0-5.0 mmol/d

mg/dL

Serum/plasma:

Newborn:	1.5-2.2 mg/dL
5 months-6 years:	1.7-2.3 mg/dL
6-12 years:	1.7-2.1 mg/dL
12-20 years:	1.7-2.2 mg/dL
Adults:	1.6-2.6 mg/dL
60-90 years:	1.6-2.4 mg/dL
> 90 years:	1.7-2.3 mg/dL
Urine (24 h):	72.9-121.5 mg/d

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 mmol/L*	*SD mmol/L*	*CV %*
PCCC1[b)]	0.812	0.00352	0.4
PCCC2[c)]	1.30	0.00546	0.4
Human serum 1	0.258	0.00386	1.5
Human serum 2	0.624	0.00384	0.6
Human serum 3	0.986	0.00346	0.4
Human serum 4	1.36	0.00567	0.4
Human serum 5	1.74	0.00577	0.3

Intermediate precision	*Mean mmol/L*	*SD mmol/L*	*CV %*
PCCC1[b)]	0.812	0.00940	1.2
PCCC2[c)]	1.30	0.0127	1.0
Human serum 1	0.258	0.00648	2.5
Human serum 2	0.624	0.00699	1.1
Human serum 3	0.986	0.00651	0.7
Human serum 4	1.37	0.00812	0.6
Human serum 5	1.74	0.00896	0.5

b) PreciControl ClinChem Multi 1
c) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control 1[d)]	1.73	0.0231	1.3
Control 2[d)]	3.67	0.0252	0.7
Human urine 1	1.50	0.0243	1.6
Human urine 2	2.90	0.0238	0.8
Human urine 3	4.08	0.0262	0.6
Human urine 4	5.30	0.0334	0.6
Human urine 5	9.02	0.0425	0.5

Intermediate precision	*Mean mmol/L*	*SD mmol/L*	*CV %*
Control 1[d)]	1.72	0.0302	1.8
Control 2[d)]	3.67	0.0313	0.9
Human urine 1	1.50	0.0288	1.9
Human urine 2	2.89	0.0336	1.2
Human urine 3	4.08	0.0298	0.7
Human urine 4	5.27	0.0424	0.8
Human urine 5	9.02	0.0609	0.7

d) commercially available control material

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

Magnesium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 97

Passing/Bablok[16]	Linear regression
y = 1.013x - 0.00748 mmol/L	y = 1.011x - 0.00537 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.100 and 1.96 mmol/L.

Urine

Sample size (n) = 62

Passing/Bablok[16]	Linear regression
y = 0.963x - 0.0757 mmol/L	y = 0.973x - 0.114 mmol/L
τ = 0.974	r = 0.999

The sample concentrations were between 0.670 and 11.0 mmol/L.

Magnesium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 72

Passing/Bablok[16]	Linear regression
y = 1.011x + 0.000944 mmol/L	y = 1.012x + 0.000238 mmol/L
τ = 0.979	r = 1.000

The sample concentrations were between 0.140 and 1.94 mmol/L.

Urine

Sample size (n) = 67

Passing/Bablok[16]	Linear regression
y = 1.007x + 0.00729 mmol/L	y = 1.008x + 0.00459 mmol/L
τ = 0.984	r = 1.000

The sample concentrations were between 0.610 and 10.7 mmol/L.

Magnesium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 68

Passing/Bablok[16]	Linear regression
y = 0.995x - 0.00153 mmol/L	y = 0.994x + 0.000658 mmol/L
τ = 0.995	r = 1.000

The sample concentrations were between 0.115 and 1.90 mmol/L.

Urine

Sample size (n) = 62

Passing/Bablok[16]	Linear regression
y = 0.994x + 0.0290 mmol/L	y = 0.992x + 0.0298 mmol/L
τ = 0.9996	r = 1.000

The sample concentrations were between 0.674 and 10.8 mmol/L.

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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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

Na Electrode

cobas®

Sodium

Order information

REF	CONTENT	Analyzer(s) on which the electrode can be used
10825468001	Na Electrode 1 (electrode)	**cobas c** 311 analyzer **cobas** 6000 analyzer series: **cobas c** 501 module **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module **cobas pure** integrated solutions: **cobas c** 303 analytical unit **cobas pro** integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Materials required (but not provided):

03149501001	REF Electrode (1 electrode)	
11360981216	ISE Reference Electrolyte (5 x 300 mL) ①②	
10820652216	ISE Reference Electrolyte (1 x 500 mL) ③④	
08392013190	ISE Reference Electrolyte (2 x 2000 mL) ⑤⑥	
04522320190	ISE Internal Standard Gen.2 (5 x 600 mL) ①②	
04880455190	ISE Internal Standard Gen.2 (2 x 2000 mL) ③④⑤	
09137742190	ISE Internal Standard Gen.2 conc. (1 x 510 mL) ⑥	
05979854190	Internal Standard Insert - ISE (Set of 20) ①②	
04522630190	ISE Diluent Gen.2 (5 x 300 mL) ①②	
04880480190	ISE Diluent Gen.2 (2 x 2000 mL) ③④⑤	
11298500316	ISE Cleaning Solution (5 x 100 mL)	
20763071122	ISE Deproteinizer (6 x 21 mL) ④⑤⑥	
03110435180	Deproteinizer (1 x 125 mL) ⑥	
04663632190	Activator (9 x 12 mL)	
11183974216	ISE Standard Low (10 x 3 mL)	Code 20502
11183982216	ISE Standard High (10 x 3 mL)	Codes 20503, 20763
12149435122	Precinorm U Plus (10 x 3 mL)	Code 20300
12149443122	Precipath U Plus (10 x 3 mL)	Code 20301
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392

ISE reagents on:

① **cobas c** 311 analyzer

② **cobas** 6000 analyzer series: **cobas c** 501 module

③ **cobas** 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

④ **cobas pure** integrated solutions: **cobas c** 303 analytical unit

⑤ **cobas pro** integrated solutions: **cobas pro** ISE analytical unit

⑥ **cobas pro** integrated solutions: **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

English

System information

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE NA	**ISE NA-U**	**ISE NA-P**	**ISE NA-S**
cobas c 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module	989	989	---	---

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	ISE NA	**ISE NA-U**	**ISE NA-P**	**ISE NA-S**
cobas c 303 analytical unit, **cobas pro** ISE analytical unit	29070	29071	29072	29073

Na Electrode

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Sodium

	ACN (Serum/ plasma)	ACN (Urine)	ACN (Plasma)	ACN (Serum)
	NA	**NA-U**	**NA-P**	**NA-S**
cobas ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit	29230	29231	29232	29233

Intended use

The Na Electrode is a device intended for the in-vitro quantitative determination of sodium in human origin serum, plasma and urine.

Summary[1]

Electrolytes are involved in most major metabolic functions in the body.

Sodium is the major extracellular cation and functions to maintain fluid distribution and osmotic pressure.

Some causes of decreased levels of sodium include prolonged vomiting or diarrhea, diminished reabsorption in the kidney and excessive fluid retention. Common causes of increased sodium include excessive fluid loss, high salt intake, and increased kidney reabsorption.

Test principle

Ion-selective electrode, using automatically diluted serum/plasma or urine specimens. The sodium electrode is based on a neutral carrier.[2,3]

Calculation

The equation given below is used for the calculation of sample and/or QC results:

$$C_S = C_{IS} \times 10^{\frac{E_S - E_{IS}}{\pm S}}$$

Where:

C_S	concentration of the ion in the sample
C_{IS}	concentration of the ion in the ISE Internal Standard
E_S	EMF of the sample
E_{IS}	EMF of the ISE Internal Standard
S	Slope of the electrode

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration.

Precautions and warnings

For in vitro diagnostic use for trained laboratory technicians.

Warning

- Samples containing material of human origin are potentially infectious. Wear personal protective equipment when replacing or installing electrodes at analyzers. If any biohazardous material is spilled, wipe it up immediately and apply a disinfectant.
- If sample or waste contacts with your skin, wash the affected area immediately with soap and water, then apply a disinfectant. Consult a physician.
- When disposing of used electrodes, treat them as biohazardous.

Caution

- Do not use electrodes after the shelf life or on-board stability period has expired. Otherwise, it may lead to unstable sodium, potassium, and chloride results due to the unstable potential reading of electrodes.
- In case of lower or higher concentration of sodium results (hypo- or hypernatremia) caused by altered lipid and/or protein content of patients' samples, rerun and/or sample checking may be necessary. Altered lipid and/or protein levels in human blood may falsely shift sodium results into the opposite direction.
- Perform electrode flow path cleaning as stated in the Instructions for Use for applicable analyzers, at the end of a daily sample run. Improper electrode flow path cleaning may cause unstable reading of electrodes and it results in calibration failures.

As with any diagnostic test procedure, results should be interpreted taking all other test results and the clinical status of the patient into consideration.

In addition, pay attention to all precautions and warnings listed in the operator's manual of the analyzer.

NOTE: Boric acid (CAS Registry No. 10043-35-3) is contained in the gel solution inside the electrode at 0.2 % of the total weight as a preservative.

Storage and stability

Store at 7-40 °C.

See labels for expiration dates.

On-board stability

After installation the electrode is stable for the following time period:
2 months or 9000 tests, whichever comes first.

The electrodes should be replaced after this time period has expired. For replacement refer to instructions in the operator's manual of the applicable analyzers.

NOTE: When replacing the electrode in **cobas pro** or **cobas pure**, the user should scan the barcode affixed on the rear side of the package instead of the barcode placed on the product's label.

Slope range 50 to 68 mV/dec

NOTE: The slope ranges for newly installed electrodes should be in the upper half of the recommended electrode slope range.

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.

It is important to follow tube manufacturers recommended procedures at and after blood collection.

Separate from cells if analysis is not performed within 4 hours.[4]

Serum

For sodium determinations, serum is the specimen of choice.

CAUTION: Serum separator tubes have to be used in accordance with the tube manufacturer's recommended procedures. If these procedures are not considered, it is possible to coat the sample probe with gel (interfering with proper sample level detection), or even to aspirate gel into the ISE system (resulting in a clogged system).

Plasma: Lithium heparin plasma

CAUTION: Inadequate mixing of plasma tubes can cause introduction of fibrin microclots into and subsequent clogging of the ISE.

NOTE: It is strongly recommended to avoid silicone-type gels, due to risk of silicon oil contaminations. In addition, tubes that exhibit a layer of clear liquid, which rises to the top of the serum after centrifugation, should not be used, in order to prevent coating the sample probes and interfering with ISE system. It is possible to clog the sample probes or the ISE tubing with gel or clots if these precautions are not taken.

Urine: Collect 24-hour urine without addition of preservatives and/or stabilizers. Store refrigerated during collection.

NOTE: Each laboratory should establish guidelines for determining acceptability of specimens and the corrective action to be taken if a specimen is considered unacceptable. Compile a laboratory-specific guideline.

Sample stability (serum, plasma): [5]

14 days at 15-25 °C

14 days at 2-8 °C

stable at (-15)-(-25) °C

up to 10 freeze-thaw cycles possible.[6]

Sample stability (urine): [5,7]

14 days at 15-25 °C

stable at (-15)-(-25) °C

up to 6 freeze-thaw cycles possible.[8]

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

Na Electrode

Sodium

Sample stability claims were established by experimental data by the manufacturer or based on reference literature[5] and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability for its laboratory.

Materials provided

See “Order information” section

Materials required (but not provided)

See “Order information” section

General laboratory equipment

Application for serum, plasma and urine

Test definition

Serum/plasma

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 80-180 mmol/L

Analysis of sodium on ISE analytical units listed with serum and plasma specimens should yield a linear relationship from 80-180 mmol/L with a deviation from the linear line of less than 5 %.

The sample volumes given above under “Normal” are for samples, calibrators, and quality controls.

Urine

	Sample dilution	
Sample volume	*Sample*	*Diluent*
cobas c 311 analyzer, cobas c 501 module		
Normal	9.7 µL	291 µL / ISE Diluent
Decreased	6.5 µL	291 µL / ISE Diluent
cobas 8000 ISE 900 / 1800 module		
Normal	10 µL	450 µL / ISE Diluent
Increased	15 µL	450 µL / ISE Diluent
cobas c 303 analytical unit, cobas pro ISE analytical unit		
Normal	15 µL	450 µL / ISE Diluent
Decreased	10 µL	450 µL / ISE Diluent
cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit		
Normal	15 µL	450 µL / System Water
Decreased	10 µL	450 µL / System Water

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit:* 20-250 mmol/L

Analysis of sodium on ISE analytical units listed with urine specimens should yield a linear relationship from 20-250 mmol/L with a deviation from the linear line of less than 10 %.

Determine samples having higher concentrations via the rerun function. Dilution of samples via rerun function is a 1:46 dilution. Results from samples diluted using the rerun function are automatically multiplied by the dilution factor.

Measuring range on *cobas c 311 analyzer, cobas c 501 module, cobas c 303 analytical unit, cobas pro ISE analytical unit, cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit* for urine samples with decreased sample volume (Rerun): 251-375 mmol/L.

Analysis of sodium on ISE analytical units listed with urine specimens should yield a linear relationship from 251-375 mmol/L with a deviation from the linear line of less than 10 %.

The sample volumes given above under “Normal” are for samples, calibrators, and quality controls.

Measuring range on *cobas 8000 ISE 900 / 1800 module:* 60-350 mmol/L

Analysis of sodium on **cobas** 8000 ISE 900 / 1800 module with urine specimens should yield a linear relationship from 60-350 mmol/L with a deviation from the linear line of less than 10 %.

Determine samples having lower concentrations via the rerun function. Dilution of samples via rerun function is a 1:31 dilution. Results from samples diluted using the rerun function are automatically multiplied by the dilution factor.

Measuring range on *cobas 8000 ISE 900 / 1800 module* for urine samples with increased sample volume (Rerun): 20-59.9 mmol/L

Analysis of sodium on **cobas** 8000 ISE 900 / 1800 module with urine specimens should yield a linear relationship from 20-59.9 mmol/L with a deviation from the linear line of less than 10 %.

The sample volumes given above under “Normal” are for samples and quality controls.

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

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 mmol/L
Limit of Detection	= 10 mmol/L
Limit of Quantitation	= 20 mmol/L

The Limit of Blank, the Limit of Detection and the 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 30 %. It has been determined using low concentration sodium samples.

Values below Limit of Quantitation are not reliable due to possible higher uncertainty.

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.

Calibration

Calibration requires the following calibrators: ISE Standard Low (S1), ISE Standard High (S2), and ISE Standard High (S3).

The slope of the calibration curve is calculated from Standards 1 and 2. ISE Internal Standard / ISE Internal Standard conc. is measured to provide E_{IS} for all measurements. Refer to the operator’s manual of the analyzer for detailed calibration instructions.

Traceability: ISE Standard Low and ISE Standard High are prepared gravimetrically from highly purified inorganic salts.

Na Electrode

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Sodium

Purity of these salts has been certified by argentometric titration, acidimetric titration or perchloric acid titration.

Calibration frequency

Calibration

- every 24 hours
- after ISE washing and maintenance
- after changing the reagent bottle ①
- after changing ISE Reference Electrolyte and/or Internal Standard conc. (depending on AutoCal settings) ②
- after replacing any electrode
- as required following quality control procedures

ISE reagents on:

① **cobas c** 311 analyzer, **cobas c** 501 module, **cobas** 8000 ISE 900 / 1800 module, **cobas c** 303 analytical unit, **cobas pro** ISE analytical unit

② **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Refer to the operator's manual for a detailed description of the Calibration/AutoCal function.

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
	Precinorm U Plus, Precipath U Plus
Urine:	Quantitative urine controls are recommended for routine quality control.

Quality controls should be performed daily and after every additional calibration.

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.

Refer to appropriate value sheets/package inserts for additional information.

Traceability: Each Roche Diagnostics control listed above has been standardized against ISE Standard Low and ISE Standard High.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Hemolysis - serum/plasma

Hemolysis:[9] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Hemolysis - urine

Hemolysis:[9] No significant interference up to a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Icterus - serum/plasma

Icterus:[9] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Lipemia - serum/plasma

Lipemia (Intralipid, SMOFlipid):[9] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Pseudohyponatremia may be seen with lipemic specimens as a result of fluid displacement.[10]

Altered protein-/lipid levels may falsely shift sodium results into the opposite direction; i.e. elevated protein level = pseudohyponatremia, decreased protein level = pseudohypernatremia.[11,12]

NOTE: Gross lipemia causes pseudohyponatremia. Grossly lipemic specimens should be cleared by ultracentrifugation.[10]

Drugs

The following drugs have been tested and caused no significant interference when added to aliquots of pooled normal human serum up to the indicated concentration.

Serum/plasma

Acetaminophen (Paracetamol)	200 mg/L
Acetylsalicylic acid	1000 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Cefoxitin	2500 mg/L
Cyclosporine	5 mg/L
Doxycyclin	50 mg/L
Heparin	5000 IU/L
Ibuprofen	500 mg/L
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
N-Acetylcysteine	1660 mg/L
Phenylbutazone	400 mg/L
Rifampicin	60 mg/L
Theophylline	100 mg/L

Urine

Acetaminophen (Paracetamol)	3000 mg/L
Ascorbic acid	4000 mg/L
Cefoxitin	12000 mg/L
Gentamycine sulfate	400 mg/L
Ibuprofen	4000 mg/L
Levodopa	1000 mg/L
Methyldopa	2000 mg/L
N-Acetylcysteine	10 mg/L
Ofloxacine	900 mg/L
Phenazopyridine	300 mg/L
Salicyluric acid	6000 mg/L
Tetracycline	300 mg/L

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.

Expected values[13]

Serum, Plasma	Infant	139-146 mmol/L
	Child	138-145 mmol/L
	Adult	136-145 mmol/L
	>90 y	132-146 mmol/L
Urine 24 h	6-10 y, M	41-115 mmol/24 h
	6-10 y, F	20-69 mmol/24 h
	10-14 y, M	63-177 mmol/24 h

Na Electrode

Sodium

10-14 y, F	48-168 mmol/24 h
Adult, M	40-220 mmol/24 h
Adult, F	27-287 mmol/24 h

The urinary excretion of sodium varies significantly with dietary intake. The values given here are typical of people on an average diet.

NOTE: It is recommended that each laboratory establishes and maintains its own reference ranges. The values given here are only to be used as a guideline.

Precision

see precision data of the following analyzers in "Appendix 1: Precision":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Method comparison

see method comparison data of the following analyzers in "Appendix 2: Method comparison":

cobas c 311 analyzer

cobas 6000 analyzer series: **cobas c** 501 module

cobas 8000 modular analyzer series: **cobas** 8000 ISE 900 / 1800 module

cobas pure integrated solutions: **cobas c** 303 analytical unit

cobas pro integrated solutions: **cobas pro** ISE analytical unit, **cobas** ISE neo 900 analytical unit, **cobas** ISE neo 1800 analytical unit

Maintenance

ISE washing procedure for cobas c 311 analyzer, cobas c 501 module, cobas 8000 ISE 900 / 1800 module, cobas c 303 and cobas pro ISE analytical unit.

The system maintenance procedures and frequencies stated in the operator's manual of the respective analyzer must be performed each day at the end of the daily sample run or after an elevated sample throughput.

cobas c 311:	The specially designated positions on the sample disk are used.
Position W1:	ISE Cleaning Solution
Position W2:	Activator

The ISE Wash procedure has to be manually selected out of maintenance items.

cobas c 501:	The specially labeled wash rack (green) is used.
Position 1:	Multiclean (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas 8000 ISE:	The specially labeled wash rack (green) is used.
Position 1:	Cell Cleaning Solution (not necessary when only the ISE is cleaned)
Position 2:	ISE Cleaning Solution
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

cobas c 303, cobas pro ISE:	The specially labeled wash rack (green) is used.
Position 1:	ISE Cleaning Solution (used for weekly wash rack)
Position 2:	ISE Cleaning Solution (used for daily wash rack)
Position 3:	Activator

The system recognizes the wash rack and switches automatically to cleaning mode.

The ISE systems require conditioning after cleaning and prior to calibration.

NOTE: Always use fresh solutions for cleaning.

ISE washing procedure for cobas ISE neo analytical unit

cobas ISE neo:	The ISE system wash tube holder is used.
Position CS:	ISE Cleaning Solution
Position A:	Activator

The maintenance task "ISE system wash" is scheduled and initiated automatically. For detailed description, refer to the operator's manual.

On-board stability of auxiliary reagents: ISE Cleaning Solution 4 days, Activator 4 days.

NOTE: Always exchange the tubes on the ISE tube holder, using new tubes for fresh reagents. **You must not refill them**, as this will lead to deterioration of the ISE measuring unit(s). Refer to the operator's manual for further information.

Appendix 1: Precision

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

	Repeatability			Intermediate precision		
Sample (on a **cobas c** 501)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	84.8	0.2	0.3	84.8	1	1.1
Plasma medium	121.4	0.3	0.3	121.4	0.8	0.6
Plasma high	176.7	0.3	0.2	176.7	0.6	0.4
Precinorm U	126	0.2	0.2	126.0	0.7	0.6
Precipath U	148.2	0.3	0.2	148.2	0.5	0.4
Urine low	30.6	0.1	0.2	30.6	0.9	3.0
Urine medium	131.7	0.2	0.2	131.7	0.6	0.5
Urine high	236.7	0.4	0.2	236.7	1.3	0.6
Liquichek 1	81.6	0.2	0.2	81.6	1.3	1.6
Liquichek 2	172.3	0.2	0.1	172.3	2.6	1.5

cobas 8000 modular analyzer series: cobas 8000 ISE 900 / 1800 module

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Na Electrode

Sodium

	Repeatability			Intermediate precision		
Sample (on a **cobas** 8000)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
Plasma low	88.7	0.3	0.4	88.7	0.9	1.1
Plasma medium	120.6	0.4	0.3	120.6	0.9	0.7
Plasma high	175.8	0.6	0.3	175.8	1.0	0.6
Precinorm U	112.0	0.4	0.4	112.0	0.9	0.8
Precipath U	144.0	0.4	0.3	144.0	0.8	0.5
Urine low[1)]	24.7	0.2	0.9	24.7	0.9	3.7
Urine medium[2)]	174.5	0.5	0.3	174.5	1.1	0.7
Urine high[2)]	347.2	0.9	0.3	347.2	2.8	0.8
Liquichek 1[2)]	83.4	0.3	0.3	83.4	1.3	1.6
Liquichek 2[2)]	175.6	1.3	0.8	175.6	1.7	1.0

1) Data obtained with urine rerun function.

2) Data obtained with default urine mode.

cobas pure integrated solutions: cobas c 303 analytical unit

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

cobas pro integrated solutions: cobas pro ISE analytical unit

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 pro** ISE analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas pro** ISE analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	111	0.36	0.3	111	0.97	0.9
PCCC2[b)]	134	0.40	0.3	134	0.90	0.7
Human plasma 1	84.7	0.28	0.3	84.7	1.25	1.5
Human plasma 2	129	0.45	0.3	129	0.88	0.7
Human plasma 3	135	0.52	0.4	135	0.93	0.7
Human plasma 4	149	0.52	0.3	149	0.82	0.6
Human plasma 5	174	0.62	0.4	174	0.95	0.5
Human serum 1	83.0	0.29	0.3	83.0	1.38	1.7
Human serum 2	131	0.52	0.4	131	0.93	0.7
Human serum 3	135	0.47	0.3	135	1.02	0.8
Human serum 4	150	0.52	0.3	150	0.80	0.5
Human serum 5	173	0.63	0.4	173	0.95	0.5
Liquichek 1	78.1	0.34	0.4	78.1	1.06	1.4
Liquichek 2	175	0.71	0.4	175	1.05	0.6
Human urine 1	24.8	0.25	1.0	24.8	1.19	4.8
Human urine 2	136	0.47	0.3	136	0.94	0.7
Human urine 3	111	0.38	0.3	111	0.94	0.8
Human urine 4	204	0.96	0.5	204	1.23	0.6
Human urine 5	241	0.95	0.4	241	1.63	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

cobas pro integrated solutions: *cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit*

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 ISE neo** analytical unit.

	Repeatability			Intermediate precision		
Sample (on a **cobas** ISE neo analytical unit)	Mean mmol/L	SD mmol/L	CV %	Mean mmol/L	SD mmol/L	CV %
PCCC1[a)]	116	0.71	0.6	116	1.40	1.2
PCCC2[b)]	139	0.83	0.6	140	1.41	1.0
Human serum 1	87.0	0.40	0.5	86.5	1.49	1.7
Human serum 2	132	0.82	0.6	132	1.07	0.8
Human serum 3	136	0.79	0.6	137	0.98	0.7
Human serum 4	159	0.80	0.5	159	1.24	0.8
Human serum 5	176	0.81	0.5	175	1.30	0.7
Human plasma 1	88.1	0.39	0.4	87.8	1.52	1.7
Human plasma 2	131	0.82	0.6	131	1.36	1.0
Human plasma 3	136	0.73	0.5	136	1.21	0.9
Human plasma 4	156	0.74	0.5	157	1.38	0.9
Human plasma 5	173	0.82	0.5	173	1.57	0.9
Liquichek 1	81.1	0.38	0.5	81.1	1.17	1.4
Liquichek 2	171	0.95	0.6	171	1.72	1.0
Human urine 1	27.0	0.30	1.1	26.1	1.20	4.6
Human urine 2	135	0.49	0.4	135	1.33	1.0
Human urine 3	111	0.43	0.4	111	1.02	0.9
Human urine 4	198	0.85	0.4	198	2.04	1.0
Human urine 5	237	1.02	0.4	237	2.84	1.2

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

Appendix 2: Method comparison

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

cobas c 311 analyzer

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

cobas 6000 analyzer series: cobas c 501 module

ISE values for human plasma and urine samples obtained on **cobas c** 501 analyzers (y) using ISE Standard High (compensated) as S3 Calibrator, were compared to those determined with the corresponding reference method (x) and with a **cobas c** 501 analyzer using ISE Compensator as S3 Calibrator.

The reference method used was: Flame Photometer IL 943 for sodium.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[14]	Coeff. (r)
x: flame photom. y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 103	86.7	178	y = 1.000x + 0.300	0.999
Bias at 135 mmol/L = 0.03 (0.2 %) Bias at 150 mmol/L = 0.03 (0.2 %)					

Na Electrode

Sodium

x: **cobas c** 501 (S3 = ISE Compensator) y: **cobas c** 501 (S3 = ISE Standard High)	Plasma / 103	87.6	176	y = 1.014x - 1.176	1.000
Bias at 135 mmol/L = 0.714 (0.5 %) Bias at 150 mmol/L = 0.924 (0.6 %)					
x: flame photom. y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 100	23.5	250	y = 0.964x + 4.032	1.000
Bias at 20 mmol/L = 3.312 (16.6 %) Bias at 220 mmol/L = -3.888 (-1.8 %)					
x: **cobas c** 501 (S3 = ISE Compensator) y: **cobas c** 501 (S3 = ISE Standard High)	Urine / 100	25.1	245	y = 0.995x + 0.687	1.000
Bias at 20 mmol/L = 0.587 (2.9 %) Bias at 220 mmol/L = -0.413 (-0.2 %)					

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas 8000 modular analyzer series:cobas 8000 ISE 900 / 1800 module

ISE values for human plasma and urine samples obtained on a **cobas** 8000 analyzer (y) using ISE Standard High as S3 Calibrator, were compared with those determined using the corresponding reference method (x) and with **cobas c** 501 (x) using ISE Standard High as S3 Calibrator.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[14]	Coeff. (r)
x: flame photom. y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	85.6	180.6	y = 1.015x - 3.553	0.9943
Bias at 135 mmol/L = -1.528 (-1.1 %) Bias at 150 mmol/L = -1.303 (-0.9 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Plasma / 100	81.5	181.9	y = 0.969x + 3.381	0.9984
Bias at 135 mmol/L = -0.804 (-0.6 %) Bias at 150 mmol/L = -1.269 (-0.8 %)					
x: flame photom. y: **cobas** 8000 (S3 = ISE Standard High)	Urine[2] / 105	69.2	337.4	y = 0.996x + 1.248	0.9995
Bias at 60 mmol/L = 1.008 (1.7 %) Bias at 220 mmol/L = 0.368 (0.2 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Urine[2] / 105	68.3	349.5	y = 0.969x + 8.259	0.9998
Bias at 60 mmol/L = 6.339 (10.7 %) Bias at 220 mmol/L = 1.439 (0.7 %)					
x: flame photom. y: **cobas** 8000 (S3 = ISE Standard High)	Urine[1] / 92	22.2	58.7	y = 0.943x + 3.149	0.9991
Bias at 30 mmol/L = 1.439 (4.8 %)					
x: **cobas c** 501 (S3 = ISE Standard High) y: **cobas** 8000 (S3 = ISE Standard High)	Urine[1] / 92	24.2	59.8	y = 0.962x + 1.110	0.9995
Bias at 30 mmol/L = - 0.03 (-0.1 %)					

1) Data obtained with urine rerun function.

2) Data obtained with default urine mode.

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pure integrated solutions: cobas c 303 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas c** 303 ISE unit (y) were compared with those determined using the corresponding reference method (x) (sodium only), with a **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas c** 303 ISE unit (y) were compared with those determined using the corresponding reference method (x) (sodium only), with a **cobas pro** ISE analytical unit (x) and with a **cobas c** 501 analyzer (x).

The reference method used was: Flame Photometer FP 8400 for sodium.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[14]	Coeff. (r)
x: flame photom. y: **cobas c** 303 ISE	Plasma / 118	81.6	176	y = 0.985x + 1.38	0.994
Bias at 135 mmol/L = -0.581 (-0.4 %) Bias at 155 mmol/L = -0.871 (-0.6 %)					
x: **cobas pro** ISE y: **cobas c** 303 ISE	Plasma / 119	84.5	174	y = 0.980x + 2.38	0.999
Bias at 135 mmol/L = -0.256 (-0.2 %) Bias at 155 mmol/L = -0.647 (-0.4 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Plasma / 119	85.8	175	y = 1.000x - 1.20	0.999

Na Electrode

Sodium

Bias at 135 mmol/L = -1.20 (-0.9 %) Bias at 155 mmol/L = -1.20 (-0.8 %)					
x: flame photom. y: **cobas c** 303 ISE	Serum / 120	81.5	182	y = 1.007x - 1.19	0.996
Bias at 135 mmol/L = -0.307 (-0.2 %) Bias at 155 mmol/L = -0.176 (-0.1 %)					
x: **cobas pro** ISE y: **cobas c** 303 ISE	Serum / 120	81.6	178	y = 0.984x + 1.23	1.000
Bias at 135 mmol/L = -0.998 (-0.7 %) Bias at 155 mmol/L = -1.32 (-0.8 %)					
x: **cobas c** 501 y: **cobas c** 303 ISE	Serum / 120	82.9	178	y = 1.000x - 1.50	1.000
Bias at 135 mmol/L = -1.50 (-1.1 %) Bias at 155 mmol/L = -1.50 (-1.0 %)					
x: flame photom. y: **cobas c** 303 ISE	Urine / 105	24.9	256	y = 0.973x + 1.97	0.999
x: **cobas pro** ISE y: **cobas c** 303 ISE	Urine / 119	19.9	246	y = 0.997x + 0.355	1.000
x: **cobas c** 501 y: **cobas c** 303 ISE	Urine / 113	22.2	237	y = 0.990x + 3.11	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas pro ISE analytical unit

ISE values for human plasma samples obtained on a **cobas pro** ISE analytical unit (y) were compared with those determined using the corresponding reference method (x) (sodium only) and with a **cobas c** 501 analyzer (x).

ISE values for human urine samples obtained on a **cobas pro** ISE analytical unit (y) were compared with those determined using the corresponding reference method (x) (sodium only) and with a **cobas c** 501 analyzer (x).

The reference method used was: Flame Photometer FP 8400 for sodium.

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[14]	Coeff. (r)
x: flame photom. y: **cobas pro** ISE	Plasma / 118	80.4	175	y = 1.031x - 4.12	0.997
Bias at 135 mmol/L = 0.037 (0.0 %) Bias at 155 mmol/L = 0.652 (0.4 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Plasma / 120	84.2	177	y = 1.003x - 1.72	1.000
Bias at 135 mmol/L = -1.33 (-1.0 %) Bias at 155 mmol/L = -1.27 (-0.8 %)					
x: flame photom. y: **cobas pro** ISE	Serum / 120	81.3	174	y = 1.016x - 1.11	0.996
Bias at 135 mmol/L = 1.09 (0.8 %) Bias at 155 mmol/L = 1.41 (0.9 %)					
x: **cobas c** 501 y: **cobas pro** ISE	Serum / 120	84.4	175	y = 1.027x - 4.38	1.000
Bias at 135 mmol/L = -0.766 (-0.6 %) Bias at 155 mmol/L = -0.230 (-0.1 %)					
x: flame photom. y: **cobas pro** ISE	Urine / 120	22.5	249	y = 0.993x - 2.46	1.000
x: **cobas c** 501 y: **cobas pro** ISE	Urine / 120	25.5	241	y = 1.019x - 2.90	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

cobas pro integrated solutions: cobas ISE neo 900 analytical unit, cobas ISE neo 1800 analytical unit

ISE values for human plasma and serum samples obtained on a **cobas** ISE neo analytical unit (y) were compared with those determined using the corresponding reference method (x), with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

ISE values for human urine samples obtained on a **cobas** ISE neo analytical unit (y) were compared with those determined using the corresponding reference method (x), with a **cobas c** 501 analyzer (x) and with a **cobas pro** ISE analytical unit (x).

The reference method used was: Flame Photometer (FP 8400).

Instruments	Sample Type/ N	Min.x	Max.x	P/B Regression[14]	Coeff. (r)
x: flame photom. y: **cobas** ISE neo	Serum / 117	85.4	182	y = 0.950x + 5.83	0.989
Bias at 135 mmol/L = -0.888 (-0.7 %) Bias at 155 mmol/L = -1.88 (-1.2 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Serum / 120	80.8	178	y = 1.009x - 1.31	0.999
Bias at 135 mmol/L = -0.112 (-0.1 %) Bias at 155 mmol/L = 0.0658 (0.0 %)					

Na Electrode

Sodium

x: **cobas pro** ISE y: **cobas** ISE neo	Serum / 119	80.7	178	y = 1.002x + 0.393	0.999
Bias at 135 mmol/L = 0.699 (0.5 %) Bias at 155 mmol/L = 0.744 (0.5 %)					
x: flame photom. y: **cobas** ISE neo	Plasma / 118	79.5	177	y = 0.967x + 5.07	0.987
Bias at 135 mmol/L = 0.591 (0.4 %) Bias at 155 mmol/L = -0.073 (0.0 %)					
x: **cobas c** 501 y: **cobas** ISE neo	Plasma / 118	85.0	176	y = 0.987x + 1.60	0.999
Bias at 135 mmol/L = -0.123 (-0.1 %) Bias at 155 mmol/L = -0.377 (-0.2 %)					
x: **cobas pro** ISE y: **cobas** ISE neo	Plasma / 119	80.5	176	y = 1.000x + 0.500	0.999
Bias at 135 mmol/L = 0.500 (0.4 %) Bias at 155 mmol/L = 0.500 (0.3 %)					
x: flame photom. y: **cobas** ISE neo	Urine / 100	25.4	238	y = 1.009x + 0.529	0.999
x: **cobas c** 501 y: **cobas** ISE neo	Urine / 116	24.1	238	y = 1.003x + 0.262	1.000
x: **cobas pro** ISE y: **cobas** ISE neo	Urine / 118	20.9	239	y = 1.010x - 0.555	1.000

Bias at the medical decision level (MDL) was calculated as follows:

Bias [mmol/L] = intercept + (slope x MDL) - MDL

Bias [%] = (Bias [mmol/L] x 100) / MDL

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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):

Cont.	Quantity contained in the package
CONTENT	Quantity contained in the package
GTIN	Global Trade Item Number
INSTALL BEFORE	Latest date by which the electrode has to be installed on the analyzer
RoHS	Directive for the restriction of the use of certain hazardous substances in electrical and electronic equipment

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

10825468001V2.0

Na Electrode

Sodium

CE 0123

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TP2

cobas®

Total Protein Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack (s) can be used
08058652190*	08058652500	Total Protein Gen.2 (1050 tests)	System-ID 2111 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058652214*	08058652500	Total Protein Gen.2 (1050 tests)	System-ID 2111 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302	
11333127122	Precipath Protein (3 x 1 mL)	Code 20303	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

TP2: ACN 21110

Intended use

In vitro test for the quantitative determination of total protein in human serum and plasma on **cobas c** systems.

Summary

Measurements of total protein, performed with this assay in human serum or plasma, are used as aid in diagnosis and monitoring of a variety of diseases involving the liver, kidney, or bone marrow, as well as other metabolic or nutritional disorders.[1,2,3,4]

Plasma proteins are synthesized predominantly in the liver, plasma cells, lymph nodes, the spleen and bone marrow. In the course of disease the total protein concentration and also the percentage represented by individual fractions can significantly deviate from normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency).

Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma proteins can be due to a change in the percentage of 1 plasma protein fraction. Often in such cases the amount of total protein does not change. The albumin/globulin (A/G) ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, lupus erythematosus as well as in certain acute and chronic inflammations.[1,2,3,4]

Test principle[5]

Colorimetric assay

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.

$$\text{protein} + Cu^{2+} \xrightarrow{\text{alkaline solution}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration which can be determined photometrically.

Reagents - working solutions

R1 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L

R3 Sodium hydroxide: 400 mmol/L; potassium sodium tartrate: 89 mmol/L; potassium iodide: 61 mmol/L; copper sulfate: 24.3 mmol/L

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H290	May be corrosive to metals.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H412	Harmful to aquatic life with long lasting effects.

Prevention:

P264	Wash skin thoroughly after handling.
P273	Avoid release to the environment.
P280	Wear protective gloves/ eye protection/ face protection.

Response:

P337 + P313	If eye irritation persists: Get medical advice/attention.
P390	Absorb spillage to prevent material damage.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

TP2

Total Protein Gen.2

Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

Storage and stability

Shelf life at 15-25 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 weeks

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.

Plasma: Li-heparin and K_2-EDTA plasma

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.

Stability:[6]	6 days at 20-25 °C
	4 weeks at 4-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

The total protein concentration is 4 to 8 g/L lower when the sample is collected from a patient situated in the recumbent position rather than upright.[7]

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)	700/546 nm		
Reagent pipetting		Diluent (H_2O)	
R1	59 µL	18 µL	
R3	21 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	1.3 µL	25 µL	50 µL
Increased	1.3 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: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 SRM 927.

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 26 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 in the unit g/L (g/dL).

Conversion factor:	g/L x 0.1 = g/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a total protein concentration of 66 g/L (6.6 g/dL).

Icterus[8]: No significant interference up to an I index of 20 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis[8]: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid)[8]: No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Dextran: No significant interference from dextran up to a concentration of 30 mg/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[11]

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

2.0-120 g/L (0.2-12 g/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from

samples diluted using the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2.0 g/L (0.2 g/dL)
Limit of Detection	= 2.0 g/L (0.2 g/dL)
Limit of Quantitation	= 3.0 g/L (0.3 g/dL)

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 total protein samples.

Expected values

g/L

Expected values according to Josephson [12]

Adults	66-87 g/L*

* calculated by unit conversion factor

Expected values according to Tietz[13]

Umbilical cord	48-80 g/L
Premature	36-60 g/L
Newborn	46-70 g/L
1 week	44-76 g/L
7 months-1 year	51-73 g/L
1-2 years	56-75 g/L
> 3 years	60-80 g/L
Adults (ambulatory)	64-83 g/L

Expected values according to Australasian Association of Clinical Biochemists[14]

Adults	60-80 g/L

g/dL

Expected values according to Josephson [12]

Adults	6.6-8.7 g/dL

Expected values according to Tietz[13]

Umbilical cord	4.8-8.0 g/dL
Premature	3.6-6.0 g/dL
Newborn	4.6-7.0 g/dL
1 week	4.4-7.6 g/dL
7 months-1 year	5.1-7.3 g/dL
1-2 years	5.6-7.5 g/dL
> 3 years	6.0-8.0 g/dL
Adults (ambulatory)	6.4-8.3 g/dL

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.

Repeatability	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
PCCC1[a)]	49.5	0.244	0.5
PCCC2[b)]	73.0	0.358	0.5
Human serum 1	4.61	0.140	3.0
Human serum 2	32.1	0.224	0.7
Human serum 3	61.4	0.292	0.5
Human serum 4	72.9	0.394	0.5
Human serum 5	103	0.588	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	g/L	*g/L*	*%*
PCCC1[a)]	49.7	0.396	0.8
PCCC2[b)]	73.0	0.495	0.7
Human serum 1	4.83	0.196	4.1
Human serum 2	32.4	0.326	1.0
Human serum 3	61.4	0.396	0.6
Human serum 4	72.9	0.453	0.6
Human serum 5	103	0.686	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

Total protein values for human serum 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) = 74

Passing/Bablok[15]	Linear regression
y = 1.010x - 0.0180 g/L	y = 1.010x - 0.0639 g/L
τ = 0.975	r = 0.999

The sample concentrations were between 8.43 and 116 g/L.

Total protein values for human serum 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) = 74

Passing/Bablok[15]	Linear regression
y = 1.012x - 0.536 g/L	y = 1.015x - 0.785 g/L
τ = 0.979	r = 1.000

The sample concentrations were between 7.50 and 117 g/L.

Total Protein 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).

TP2

Total Protein Gen.2

Sample size (n) = 69

Passing/Bablok[15]	Linear regression
y = 1.008x - 1.25 g/L	y = 1.017x - 1.86 g/L
τ = 0.987	r = 0.999

The sample concentrations were between 7.66 and 115 g/L.

References

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3 Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 5th ed. 2012.

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5 Weichselbaum TE. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Pathol 1946;10:40-49.

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8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

9 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

10 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

12 Josephson B, Gyllenswärd C. The Development of the Protein Fractions and of Cholesterol Concentration in the Serum of Normal Infants and Children. Scandinav J Clin Lab Investigation 1957;9:29.

13 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;518-523.

14 Tate JR, Sikaris KA, Jones GRD, et al. Harmonising adult and paediatric reference intervals in Australia and New Zealand: An evidence-based approach for establishing a first panel of chemistry analytes. Clin Biochem Rev 2014; Nov 35(4):213-35.

15 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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

TRIGL

cobas®

Triglycerides

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058687190*	08058687500	Triglycerides (1000 tests)	System-ID 2113 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058687214*	08058687500	Triglycerides (1000 tests)	System-ID 2113 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

TRIGL: ACN 21130

Intended use

In vitro test for the quantitative determination of triglycerides in human serum and plasma on **cobas c** systems.

Summary

Triglyceride measurements, performed with this assay in human serum and plasma are used as an aid in identifying patients at risk of developing atherosclerosis and for the diagnosis of dyslipidemias.

Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids.[1] They are partly synthesized in the liver and partly ingested in food. Triglycerides are water-insoluble molecules and are carried in the circulation in water-soluble complexes called lipoproteins. The plasma triglyceride level reflects the concentration of the triglyceride-carrying lipoproteins VLDL (very-low-density lipoproteins) and chylomicrons.[2] Chylomicrons are primarily involved in the absorption and delivery of dietary fat while VLDLs deliver endogenous lipids to other tissues.

Triglycerides are considered a risk factor for atherosclerotic cardiovascular disease.[3] Cardiovascular risk is increased when fasting triglycerides are > 1.7 mmol/L (> 150 mg/dL). Individuals with triglycerides > 2.3 mmol/L (> 200 mg/dL) are considered at high risk. The determination of triglycerides is utilized in the diagnosis of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.[4]

Elevated levels of plasma triglycerides are also associated with an increased risk of acute pancreatitis and aortic valve stenosis.[5]

The enzymatic triglycerides assay as described by Eggstein and Kreutz still required saponification with potassium hydroxide. Numerous attempts were subsequently made to replace alkaline saponification by enzymatic hydrolysis with lipase.[6] Bucolo and David tested a lipase/protease mixture; Wahlefeld used an esterase from the liver in combination with a particularly effective lipase from Rhizopus arrhizus for hydrolysis.[7, 8]

This method is based on the work by Wahlefeld using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.[9,10]

Test principle[10]

Enzymatic colorimetric test.

$$\text{triglycerides} + 3\ H_2O \xrightarrow{\text{LPL}} \text{glycerol} + 3\ \text{RCOOH}$$

$$\text{glycerol} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2\ H_2O + HCl$$

Reagents - working solutions

R1 PIPES buffer: 50 mmol/L, pH 6.8; Mg^{2+}: 40 mmol/L; sodium cholate: 0.20 mmol/L; ATP: ≥ 1.4 mmol/L; 4-aminophenazone: ≥ 0.13 mmol/L; 4-chlorophenol: 4.7 mmol/L; lipoprotein lipase (Pseudomonas spec.): ≥ 83 µkat/L; glycerol kinase (Bacillus stearothermophilus): ≥ 3 µkat/L; glycerol phosphate oxidase (E. coli): ≥ 41 µkat/L; peroxidase (horseradish): ≥ 1.6 µkat/L; preservative, stabilizers

R1 is in position B.

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:	26 weeks

Specimen collection and preparation

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

TRIGL

cobas®

Triglycerides

Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K_2-EDTA plasma.

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.

Stability in serum:	2 days at 20-25 °C[11]
	10 days at 2-8 °C[12]
	3 months at -20 °C (± 5 °C)[13]
	several years at -70 °C (± 5 °C)[13]

Freeze only once.

Stability in plasma:	2 days at 20-25 °C[11]
	15 days at 2-8 °C[14]
	3 months at -20 °C (± 5 °C)[13]
	several years at -70 °C (± 5 °C)[13]

Freeze only once.

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)	700/505 nm	
Reagent pipetting		Diluent (H_2O)
R1	66 µL	15 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.1 µL	–	–
Decreased	1.1 µL	15 µL	60 µL
Increased	1.1 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: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 8 weeks on-board - 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 the ID/MS method.

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 26 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 in the unit mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L x 88.5 = mg/dL
	mmol/L x 0.885 = g/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a triglyceride concentration of 2.3 mmol/L (203 mg/dL).

Icterus:[15] No significant interference up to an I index of 10 for conjugated bilirubin and 10 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 171 µmol/L or 10 mg/dL; approximate unconjugated bilirubin concentration: 171 µmol/L or 10 mg/dL).

Hemolysis:[15] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 434 µmol/L or 700 mg/dL).

Lipemia:[15] The L index correlates with sample turbidity but not with triglycerides level. Extremely lipemic samples (triglycerides greater than 3000 mg/dL) can produce normal results[16].

Prozone Check: The flag > Kin is an indicator for extremely high triglyceride concentrations in the sample. False low results are due to oxygen depletion during assay reaction.

Endogenous unesterified glycerol in the sample will falsely elevate serum triglycerides.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[17,18]

Exception: Ascorbic acid and calcium dobesilate cause artificially low triglyceride results. Intralipid is directly measured as analyte in this assay and leads to high triglyceride results.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[19]

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at a plasma concentration above 166 mg/L and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results. A significant interference may occur at plasma Metamizole concentrations above 0.05 mg/mL.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[20]

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

0.1-10.0 mmol/L (8.85-885 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 mmol/L (8.85 mg/dL)
Limit of Detection	= 0.1 mmol/L (8.85 mg/dL)
Limit of Quantitation	= 0.1 mmol/L (8.85 mg/dL)

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 triglycerides samples.

Expected values according to NCEP[21]

mmol/L

Normal range: < 1.70 mmol/L

Clinical interpretation according to the recommendations of the European Atherosclerosis Society: [22]

	mmol/L	Lipid metabolism disorder
Cholesterol Triglycerides	< 5.18 < 2.26	No
Cholesterol	5.18-7.77	Yes if HDL-cholesterol < 0.9 mmol/L
Cholesterol Triglycerides	> 7.77 > 2.26	Yes

mg/dL

Normal range: < 150 mg/dL

Clinical interpretation according to the recommendations of the European Atherosclerosis Society: [22]

	mg/dL	Lipid metabolism disorder
Cholesterol Triglycerides	< 200 < 200	No
Cholesterol	200-300	Yes if HDL-cholesterol < 35 mg/dL
Cholesterol Triglycerides	> 300 > 200	Yes

Note: If the free glycerol is to be taken into account, then 0.11 mmol/L (10 mg/dL) must be subtracted from the triglycerides value obtained.[13]

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.

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	1.37	0.00824	0.6
PCCC2[b)]	2.50	0.0150	0.6
Human serum 1	0.195	0.00414	2.1
Human serum 2	1.73	0.0107	0.6
Human serum 3	3.14	0.0229	0.7
Human serum 4	5.25	0.0324	0.6
Human serum 5	8.56	0.0476	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	1.37	0.0104	0.8
PCCC2[b)]	2.51	0.0209	0.8
Human serum 1	0.195	0.00443	2.3
Human serum 2	1.73	0.0126	0.7
Human serum 3	3.14	0.0250	0.8
Human serum 4	5.23	0.0350	0.7
Human serum 5	8.50	0.0555	0.7

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

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

Triglycerides 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) = 74

Passing/Bablok[23]	Linear regression
y = 1.015x + 0.0125 mmol/L	y = 1.020x + 0.00786 mmol/L
τ = 0.983	r = 0.999

The sample concentrations were between 0.300 and 9.19 mmol/L.

Triglycerides 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) = 74

Passing/Bablok[23]	Linear regression
y = 1.019x - 0.00772 mmol/L	y = 1.021x - 0.0114 mmol/L
τ = 0.994	r = 1.000

The sample concentrations were between 0.170 and 9.63 mmol/L.

TRIGL

cobas®

Triglycerides

Triglycerides 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) = 75

Passing/Bablok[23]	Linear regression
y = 1.018x - 0.0236 mmol/L	y = 1.022x - 0.0320 mmol/L
τ = 0.995	r = 1.000

The sample concentrations were between 0.611 and 9.72 mmol/L.

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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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

UA2

cobas®

Uric Acid ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058750190*	08058750500	Uric Acid ver.2 (1300 tests)	System-ID 2117 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058750214*	08058750500	Uric Acid ver.2 (1300 tests)	System-ID 2117 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

UA2: ACN 21170 (Serum/plasma)

UA2U: ACN 21171 (Urine)

Intended use

In vitro test for the quantitative determination of uric acid in human serum, plasma and urine on **cobas c** systems.

Summary

Uric acid measurements, performed with this assay, in human serum, plasma and urine are used as aid in diagnosis and treatment of numerous renal and metabolic disorders associated with hyper- or hypo-uricemia.

Uric acid is the major final product of purine metabolism in the human organism. Purines from dietary nucleic acids are converted in the liver and small intestine to uric acid.[1] Uric acid is present as a normal intracellular component and in biological fluids. Chemically, it is a reducing agent and accounts for nearly half of the antioxidant activity in blood. Uric acid production is balanced between purine ingestion, de novo synthesis, reabsorption, and degradation. Two-thirds of uric acid is excreted renally, while one-third is eliminated through the gastrointestinal system. Serum uric acid levels increase physiologically and gradually over the course of human life and are strongly influenced by the diet.[1,2]

High serum levels of uric acid can adversely affect organ systems. Overproduction of uric acid, insufficient excretion of uric acid, or often a combination of both can lead to hyperuricemia.[3] Primary causes of hyperuricemia include idiopathic and hereditary metabolic disorders. Secondary causes of increased uric acid formation include excessive dietary intake of purines and increased nucleic acid turnover (e.g. in myeloproliferative disorders, lymphoproliferative disorders, psoriasis, sarcoidosis, hemolytic anemia, cytotoxic drug treatments). Major causes of decreased uric acid excretion are: acute or chronic kidney disease, increased renal tubular reabsorption, reduced tubular secretion, lead poisoning, preeclampsia, low doses of salicylate, thiazide diuretics, Down syndrome.[1]

Hyperuricemia is mostly asymptomatic, but persistent hyperuricemia and uric acid precipitation may lead to the accumulation of urate crystals in many tissues, resulting in either acute painful conditions, such as gout/tophaceous gout/gouty arthritis, urolithiasis, or, in severe cases, in uric acid kidney diseases.[4]

Hypouricemia is much less common than hyperuricemia. Hypouricemia is often defined as serum uric acid levels ≤ 2.0 mg/dL (0.12 mmol/L). It may be secondary to any one of a number of underlying conditions, such as severe hepatocellular disease with reduced purine synthesis or xanthine oxidase activity, defective renal tubular reabsorption of uric acid (congenital or acquired), overtreatment of hyperuricemia, treatment with uricosuric drugs and cancer chemotherapy with 6-mercaptopurine or azathioprine.[1,5]

Phosphotungstic acid (PTA), uricase, and HPLC-based methods have been described for measuring uric acid. PTA methods are now rarely used.[1,6] The uricase-based method utilizes the enzyme uricase to oxidize uric acid.[7] Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzymes to provide a colorimetric assay.[1]

The colorimetric method developed by Town, et al. involves initial sample incubation with a reagent mixture containing ascorbate oxidase and a clearing system. In this test system it is important that any ascorbic acid present in the sample is eliminated in the preliminary reaction; this precludes any ascorbic acid interference with the subsequent peroxidase (POD) indicator reaction. Upon addition of the starter reagent, oxidation of uric acid by uricase begins.[8]

The Roche assay described here is a slight modification of the colorimetric method described above. In this reaction, the peroxide reacts in the presence of peroxidase (POD), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (TOOS), and 4-aminophenazone to form a quinone-diimine dye. The intensity of the red color formed is proportional to the uric acid concentration and is determined photometrically.

Test principle

Enzymatic colorimetric test.

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$\text{Uric acid} + 2\ H_2O + O_2 \xrightarrow{\text{Uricase}} \text{allantoin} + CO_2 + H_2O_2$$

In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye.

$$2\ H_2O_2 + H^+ + \text{TOOS}^{a)} + \text{4-aminophenazone} \xrightarrow{\text{Peroxidase}} \text{quinone-diimine dye} + 4\ H_2O$$

a) N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline

The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8 %; ascorbate oxidase (EC 1.10.3.3; zucchini) ≥ 83.5 µkat/L (25 °C); stabilizers; preservative

R3 Phosphate buffer: 0.1 mol/L, pH 7.8; potassium hexacyanoferrate (II): 0.3 mmol/L; 4-aminophenazone ≥ 3 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae) ≥ 83.4 µkat/L (25 °C); peroxidase (POD) (EC 1.11.1.7; horseradish) ≥ 50 µkat/L (25 °C); stabilizers; 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H319	Causes serious eye irritation.

Prevention:

P264	Wash skin thoroughly after handling.
P280	Wear eye protection/ face protection.

Response:

P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical advice/attention.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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
	6 months at -20 °C (± 5 °C)

Freeze only once.

Stability in urine[9] (upon NaOH addition):	4 days at 20-25 °C

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.
Plasma: Li-heparin and K_2-EDTA plasma.

EDTA plasma values are approximately 7 % lower than serum values.

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.

Urine: Assay urinary uric acid as soon as possible. Do not refrigerate.

To prevent ureate precipitation in urine samples, add sodium hydroxide to keep urine alkaline (pH > 8.0). To achieve stated uric acid stability, add NaOH prior to sample collection. Urine samples are diluted 1 + 10 with distilled/deionized water or 0.9 % NaCl. This dilution is taken into account in the calculation of the results. If stabilizers are added to the sample, the sample index feature must not be used.

Centrifuge samples containing precipitates before performing the assay.

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

Stability in serum/plasma:[9]	7 days at 4-8 °C
	3 days at 20-25 °C

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)	700/546 nm	
Reagent pipetting		Diluent (H_2O)
R1	55 µL	19 µL
R3	11 µL	15 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	–	–
Decreased	3.6 µL	21 µL	61 µL
Increased	2.3 µL	–	–

Application for urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	700/546 nm	
Reagent pipetting		Diluent (H_2O)
R1	55 µL	19 µL
R3	11 µL	15 µL

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	10 µL	100 µL
Decreased	2.3 µL	4 µL	106 µL
Increased	2.3 µL	10 µL	100 µL

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

Calibration

Application for serum/plasma (ACN 21170)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear

Calibration frequency	Automatic full calibration - after reagent lot change Full calibration - every 12 weeks on-board - as required following quality control procedures

Application for urine (ACN 21171)

Transfer of calibration from serum/plasma application (ACN 21170)

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

Traceability: This method has been standardized against ID/MS.[10]

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 26 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 in the unit mg/dL (µmol/L, mg/L, mmol/L).

Conversion factors:	mg/dL x 59.5 = µmol/L
	mg/dL x 10.0 = mg/L
	mg/dL x 0.0595 = mmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a uric acid concentration of 7 mg/dL (417 µmol/L) in serum/plasma and at a uric acid concentration of 92 mg/dL (5474 µmol/L) in urine. Recovery within ± 10 % for drug interference.

Serum/plasma

Icterus:[11] No significant interference up to an I index of 40 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 684 µmol/L or 40 mg/dL).

Hemolysis:[11] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[11] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12,13] Exceptions: Calcium dobesilate causes artificially low uric acid results.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.[14]

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 0.17 mmol/L (3 mg/dL).

Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

Acetaminophen intoxications are frequently treated with N-Acetylcysteine. N-Acetylcysteine at the therapeutic concentration when used as an antidote and the Acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of Metamizole. Venipuncture immediately after or during the administration of Metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13] Exceptions: Calcium dobesilate, Levodopa and methyldopa can all cause artificially low uric acid results.

Dicynone (Etamsylate) at therapeutic concentrations may lead to false-low results.

High homogentisic acid concentrations in urine samples lead to false results.

Acetaminophen, Acetylcysteine and Metamizole are metabolized quickly. Therefore, interference from these substances is unlikely but cannot be excluded.

Hemolysis: No significant interference up to an H index of 750 (approximate hemoglobin concentration: 466 µmol/L or 750 mg/dL).

Urea: No significant interference from urea up to a concentration of 2100 mmol/L (12612 mg/dL).

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

Serum/plasma

0.2-25 mg/dL (11.9-1487 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Urine

2.2-275 mg/dL (131-16362 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma

Limit of Blank	= 0.2 mg/dL
Limit of Detection	= 0.2 mg/dL
Limit of Quantitation	= 0.2 mg/dL

Urine

Limit of Blank	= 2.2 mg/dL
Limit of Detection	= 2.2 mg/dL
Limit of Quantitation	= 2.2 mg/dL

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 uric acid samples.

Expected values
mg/dL

Serum/plasma[16]

Males: 3.4-7.0 mg/dL

Females: 2.4-5.7 mg/dL

Urine (reference range according to Krieg and Colombo)

1st morning urine[17] 37-92 mg/dL*

24-hour urine[18] 200-1000 mg/day*

corresponding to 13-67 mg/dL

(calculated from a urine volume of 1.5 L/24 h)

µmol/L

Serum/plasma[16]

Males: 202.3-416.5 µmol/L*

Females: 142.8-339.2 µmol/L*

* calculated by unit conversion factor

Urine (reference range according to Krieg and Colombo)

1st morning urine[17] 2200-5475 µmol/L

24-hour urine[18] 1200-5900 µmol/day

corresponding to 773-3986 µmol/L

(calculated from a urine volume of 1.5 L/24 h)

Urine (reference range according to Tietz)[19]

Average diet		250-750 mg/24 hours
Low purine diet		
	Females	< 400 mg/24 hours
	Males	< 480 mg/24 hours
High purine diet		< 1000 mg/24 hours

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 mg/dL*	*SD mg/dL*	*CV %*
PCCC1[b)]	4.60	0.0193	0.4
PCCC2[c)]	10.6	0.0655	0.6
Human serum 1	0.430	0.00713	1.7
Human serum 2	2.32	0.0147	0.6
Human serum 3	6.66	0.0347	0.5
Human serum 4	12.0	0.0756	0.6
Human serum 5	21.4	0.129	0.6

Intermediate precision	*Mean mg/dL*	*SD mg/dL*	*CV %*
PCCC1[b)]	4.60	0.0467	1.0
PCCC2[c)]	10.6	0.0983	0.9
Human serum 1	0.430	0.00880	2.0
Human serum 2	2.32	0.0185	0.8
Human serum 3	6.66	0.0400	0.6
Human serum 4	12.0	0.0940	0.8
Human serum 5	21.4	0.143	0.7

b) PreciControl ClinChem Multi 1
c) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean mg/dL*	*SD mg/dL*	*CV %*
Control 1[d)]	9.05	0.0780	0.9
Control 2[d)]	16.1	0.0957	0.6
Human urine 1	2.91	0.0584	2.0
Human urine 2	37.1	0.171	0.5
Human urine 3	74.7	0.279	0.4
Human urine 4	115	0.556	0.5
Human urine 5	224	0.866	0.4

Intermediate precision	*Mean mg/dL*	*SD mg/dL*	*CV %*
Control 1[d)]	9.18	0.144	1.6
Control 2[d)]	16.1	0.159	1.0
Human urine 1	3.06	0.576	18.8
Human urine 2	37.1	0.615	1.7
Human urine 3	74.9	1.93	2.6
Human urine 4	115	4.34	3.8
Human urine 5	224	1.39	0.6

d) commercially available control material

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

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

Serum/plasma

Sample size (n) = 88

Passing/Bablok[20]	Linear regression
y = 1.004x - 0.0207 mg/dL	y = 1.008x - 0.0265 mg/dL
τ = 0.985	r = 1.000

The sample concentrations were between 0.290 and 24.6 mg/dL.

Urine

Sample size (n) = 81

Passing/Bablok[20]	Linear regression
y = 1.002x - 0.168 mg/dL	y = 1.004x - 0.162 mg/dL
τ = 0.987	r = 1.000

The sample concentrations were between 3.27 and 270 mg/dL.

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

Serum/plasma

Sample size (n) = 83

Passing/Bablok[20]	Linear regression
y = 1.000x + 0.003 mg/dL	y = 1.023x - 0.141 mg/dL
τ = 0.979	r = 0.998

The sample concentrations were between 0.200 and 23.7 mg/dL.

Urine

Sample size (n) = 102

Passing/Bablok[20]	Linear regression
y = 1.038x - 0.0408 mg/dL	y = 1.057x - 0.990 mg/dL
τ = 0.991	r = 1.000

The sample concentrations were between 2.45 and 243 mg/dL.

Uric acid values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 76

Passing/Bablok[20]	Linear regression
y = 1.009x - 0.0125 mg/dL	y = 0.999x + 0.0327 mg/dL
τ = 0.963	r = 1.000

The sample concentrations were between 0.294 and 23.3 mg/dL.

Urine

Sample size (n) = 66

Passing/Bablok[20]	Linear regression
y = 0.990x - 0.0957 mg/dL	y = 0.994x - 0.220 mg/dL
τ = 0.997	r = 1.000

The sample concentrations were between 4.41 and 254 mg/dL.

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.

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

UREAL

cobas®

Urea/BUN

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08058806190*	08058806500	Urea/BUN (600 tests)	System-ID 2119 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08058806214*	08058806500	Urea/BUN (600 tests)	System-ID 2119 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

UREAL: ACN 21191 (Serum/plasma)

URELU: ACN 21190 (Urine)

U-BUN: ACN 21192 (Serum/plasma)

UBUNU: ACN 21193 (Urine)

Intended use

In vitro test for the quantitative determination of urea/urea nitrogen in human serum, plasma and urine on **cobas c** systems.

Summary

Measurements of urea/urea nitrogen in human serum, plasma and urine, performed with this assay are used as screening tests and as an aid in diagnosis and monitoring of renal function.

Urea is the major end product of protein nitrogen metabolism. It is synthesized by the urea cycle in the liver from ammonia which is produced by amino acid deamination. Urea is excreted mostly by the kidneys but minimal amounts are also excreted in sweat and degraded in the intestines by bacterial action.[1]

Serum urea mass concentration is either specified for the complete urea molecule or for nitrogen equivalents [blood urea nitrogen (BUN)].[2] Determination of blood urea nitrogen is primarily used as a screening test for renal function. When used in conjunction with serum creatinine determinations it can aid in the differential diagnosis of the three types of azotemia: prerenal, renal, and postrenal. The urea to creatinine ratio has been proposed as a crude discriminator between prerenal and intrinsic azotemia.[1]

Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (prerenal causes), chronic nephritis, nephrosclerosis, tubular necrosis, glomerular-nephritis (renal causes), and urinary tract obstruction (postrenal causes). Transient elevations may also be seen during periods of high protein intake. Liver diseases may lead to unpredictable blood urea nitrogen concentrations, including abnormally low levels. Low blood urea nitrogen concentrations are not common, but can be found in cases such as malnutrition, lack of protein in the diet, or overhydration.[1,3]

Test principle

Kinetic test with urease and glutamate dehydrogenase.[4,5,6,7]

Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3^{2-}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction 2 moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

Reagents - working solutions

R1 NaCl 9 %

R3 TRIS buffer: 220 mmol/L, pH 8.6; 2-oxoglutarate: 73 mmol/L; NADH: 2.5 mmol/L; ADP: 6.5 mmol/L; urease (jack bean): ≥ 300 µkat/L; GLDH (bovine liver): ≥ 80 µkat/L; preservative; nonreactive stabilizers

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for laboratory 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:	8 weeks

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
Plasma: Li-heparin and K_2-EDTA plasma. Do not use ammonium heparin.

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.

Urine

Bacterial growth in the specimen and high atmospheric ammonia concentrations as well as contamination by ammonium ions may cause

erroneously elevated results. If stabilizers are added to the sample, the sample index feature must not be used.

Stability in *serum/plasma*:[8]	7 days at 15-25 °C
	7 days at 2-8 °C
	1 year at (–15)-(–25) °C

Freeze only once.

Stability in *urine*:[8]	2 days at 15-25 °C
	7 days at 2-8 °C
	1 month at (–15)-(–25) °C

Freeze only once.

Centrifuge samples containing precipitates before performing the assay.

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

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	8 µL	66 µL	
R3	28 µL	81 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	25 µL	50 µL
Increased	1.5 µL	–	–

Application for urine

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	8 µL	66 µL	
R3	28 µL	81 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	2.0 µL	98 µL
Decreased	1.5 µL	1.3 µL	116 µL
Increased	1.5 µL	–	–

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

Calibration

Application for serum/plasma (ACN 21191/21192)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - every 4 weeks on-board - as required following quality control procedures

Application for urine (ACN 21190/21193)

Transfer of calibration from serum/plasma application (ACN 21191/21192)

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

Traceability: This method has been standardized against ID/MS.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 8 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 in the unit mmol/L (mg/dL, g/L).

Conversion factors:

mmol/L urea x 6.006 = mg/dL urea

mmol/L urea x 0.06006 = g/L urea

mmol/L urea nitrogen x 2.801 = mg/dL urea nitrogen

mmol/L urea nitrogen x 0.02801 = g/L urea nitrogen

mg/dL urea x 0.467 = mg/dL urea nitrogen

When 24-hour urine is used as the specimen, multiply the result by the 24-hour volume to obtain values in g or mmol/24 hours.

Limitations - interference

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

Serum/plasma

Criterion: Recovery within ± 0.83 mmol/L of initial values of samples ≤ 8.3 mmol/L and within ± 10 % for samples > 8.3 mmol/L.

Icterus:[10] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[10] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[10] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Ammonium ions may cause erroneously elevated results.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[11,12]

Urine

Criterion: Recovery within ± 15 mmol/L of initial values of samples ≤ 150 mmol/L and within ± 10 % for samples > 150 mmol/L.

Hemolysis: No significant interference up to an H index of 750 (approximate hemoglobin concentration: 466 µmol/L or 750 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12]

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

Serum/plasma

0.5-40 mmol/L (3.0-240 mg/dL urea, 1.4-112 mg/dL urea nitrogen)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Urine

1-2000 mmol/L (6-12000 mg/dL urea, 2.8-5600 mg/dL urea nitrogen)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.8 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 1.8.

Determine samples having concentrations lower than the technical limit of 40 mmol/L (240 mg/dL urea and 112 mg/dL urea nitrogen) via the rerun function. Samples are measured undiluted.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma

Limit of Blank	= 0.5 mmol/L
Limit of Detection	= 0.5 mmol/L
Limit of Quantitation	= 0.5 mmol/L

Urine

Limit of Blank	= 1.0 mmol/L
Limit of Detection	= 1.0 mmol/L
Limit of Quantitation	= 1.0 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 urea/urea nitrogen samples.

Expected values

mmol/L

Urea:

Serum/plasma[13]

Adults	2.76-8.07 mmol/L
Urine	
24-hour urine[14]	428-714 mmol/24 h, corresponding to 286-595 mmol/L[a)]

a) Based on average urine output of 1.2-1.5 L/24 h

Urea nitrogen (BUN):

Serum/plasma[14]

Adults (18-60 years)	2.14-7.14 mmol/L
Adults (60-90 years)	2.86-8.21 mmol/L
Infants (< 1 year)	1.43-6.78 mmol/L
Infants/children	1.79-6.43 mmol/L
Urine	
24-hour urine[14]	428-714 mmol/24 h, corresponding to 286-595 mmol/L[b)]

b) Based on average urine output of 1.2-1.5 L/24 h

mg/dL

Urea:

Serum/plasma[13]

Adults	16.6-48.5 mg/dL
Urine	
24-hour urine[14]	25.7-42.9 g/24 h, corresponding to 1.71-3.57 g/dL[a)]

a) Based on average urine output of 1.2-1.5 L/24 h

Urea nitrogen (BUN):

Serum/plasma[14]

Adults (18-60 years)	6-20 mg/dL
Adults (60-90 years)	8-23 mg/dL
Infants (< 1 year)	4-19 mg/dL
Infants/children	5-18 mg/dL
Urine	
24-hour urine[14]	12-20 g/24 h, corresponding to 801-1666 mg/dL[b)]

b) Based on average urine output of 1.2-1.5 L/24 h

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

UREAL

cobas®

Urea/BUN

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[c)]	6.53	0.0408	0.6
PCCC2[d)]	18.3	0.0690	0.4
Human serum 1	1.31	0.0416	3.2
Human serum 2	5.12	0.0441	0.9
Human serum 3	7.67	0.0451	0.6
Human serum 4	18.7	0.101	0.5
Human serum 5	31.0	0.124	0.4

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[c)]	6.50	0.0745	1.1
PCCC2[d)]	18.4	0.198	1.1
Human serum 1	1.31	0.0459	3.5
Human serum 2	5.12	0.0659	1.3
Human serum 3	7.67	0.0931	1.2
Human serum 4	18.7	0.226	1.2
Human serum 5	31.0	0.350	1.1

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Control 1[e)]	143	2.86	2.0
Control 2[e)]	239	3.68	1.5
Human urine 1	3.22	0.0435	1.4
Human urine 2	73.2	2.50	3.4
Human urine 3	407	3.28	0.8
Human urine 4	922	5.03	0.5
Human urine 5	1583	10.1	0.6

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Control 1[e)]	143	3.17	2.2
Control 2[e)]	239	4.32	1.8
Human urine 1	3.22	0.0547	1.7
Human urine 2	73.2	2.78	3.8
Human urine 3	411	4.93	1.2
Human urine 4	919	11.5	1.2
Human urine 5	1583	19.7	1.2

e) commercially available control material

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

Urea values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 94

Passing/Bablok[15]	Linear regression
y = 1.009x + 0.0202 mmol/L	y = 1.006x + 0.0265 mmol/L
τ = 0.986	r = 1.000

The sample concentrations were between 0.600 and 38.1 mmol/L.

Urine

Sample size (n) = 91

Passing/Bablok[15]	Linear regression
y = 0.962x - 0.432 mmol/L	y = 0.960x + 0.586 mmol/L
τ = 0.982	r = 1.000

The sample concentrations were between 71.0 and 1964 mmol/L.

Urea values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 89

Passing/Bablok[15]	Linear regression
y = 1.017x + 0.0905 mmol/L	y = 1.015x + 0.148 mmol/L
τ = 0.986	r = 1.000

The sample concentrations were between 0.700 and 35.4 mmol/L.

Urine

Sample size (n) = 73

Passing/Bablok[15]	Linear regression
y = 0.981x + 0.901 mmol/L	y = 0.973x + 4.74 mmol/L
τ = 0.960	r = 0.999

The sample concentrations were between 41.0 and 1875 mmol/L.

Urea values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 74

Passing/Bablok[15]	Linear regression
y = 1.000x + 0.0800 mmol/L	y = 1.000x + 0.0683 mmol/L
τ = 0.983	r = 1.000

The sample concentrations were between 0.821 and 39.5 mmol/L.

Urine

Sample size (n) = 74

Passing/Bablok[15]	Linear regression
y = 0.948x - 1.68 mmol/L	y = 0.942x + 0.489 mmol/L
τ = 0.980	r = 1.000

The sample concentrations were between 46.4 and 1912 mmol/L.

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15 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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

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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