CO2-L

cobas®

Bicarbonate Liquid

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057494190	Bicarbonate Liquid (250 tests)	System-ID 2044 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

20751995190	Ammonia/Ethanol/CO2 Calibrator (2 x 4 mL)	Code 20688	
20752401190	Ammonia/Ethanol/CO2 Control Normal (5 x 4 mL)	Code 20100	
20753009190	Ammonia/Ethanol/CO2 Control Abnormal (5 x 4 mL)	Code 20101	

English

System information

CO2-L: ACN 20440

Intended use

In vitro test for the quantitative determination of bicarbonate (HCO_3^-) in human serum and plasma on **cobas c** systems.

Summary

Bicarbonate is the second largest fraction of the anions in plasma. Included in this fraction are the bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, as well as the carbamino compounds. At the physiological pH of blood, the concentration of carbonate is 1/1000 that of bicarbonate. The carbamino compounds are also present in such low quantities that they are generally not mentioned specifically.

Several different methods for the determination of bicarbonate in serum and plasma have been reported. Most of these procedures utilize acidification of the sample and conversion of all carbon dioxide forms to CO_2 gas.[1] The amount of gas formed is measured by manometric or volumetric devices, ion selective electrodes, or spectrophotometric techniques.[2,3] These methods are either cumbersome, time-consuming, technique-oriented, and/or require special equipment.

Enzymatic procedures using phosphoenolpyruvate carboxylase (PEPC) have been described.[4,5]

The bicarbonate content of serum or plasma is a significant indicator of electrolyte dispersion and anion deficit. Together with pH determination, bicarbonate measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with acid-base imbalance in the respiratory and metabolic systems.

Test principle

Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPC to produce oxaloacetate and phosphate:

$$PEP + HCO_3^- \xrightarrow{PEPC} \text{oxaloacetate} + H_2PO_4^-$$

The above reaction is coupled with one involving the transfer of a hydrogen ion from NADH analog to oxaloacetate using MDH.

$$\text{Oxaloacetate} + \text{NADH analog} + H^+ \xrightarrow{MDH} \text{malate} + NAD^+ \text{ analog}$$

The resultant consumption of NADH analog causes a decrease in absorbance, which is proportional to the concentration of bicarbonate in the sample being assayed.

Reagents - working solutions

R1 Phosphoenolpyruvate: ≥ 40 mmol/L; NADH analog: ≥ 2 mmol/L; MDH (porcine): ≥ 314.3 µkat/L; PEPC (microbial): ≥ 30.8 µkat/L

R1 is in position C.

Precautions and warnings

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

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

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 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 preferred specimen is from venous blood collected anaerobically in the usual manner for bicarbonate analysis. Bicarbonate content in uncapped tubes decreases approximately 4 mmol/L after one hour.[6] It has been reported that alkalinized serum stored in open cups is stable for up to 4 hours.[6]

Storage of serum at -20 °C or -80 °C for up to 6 months had no significant effect.[7]

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:	7 days at 4-8 °C[8]
	40 hours at 15-25 °C[9,10]

Separate from erythrocytes and store tightly stoppered.

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)	505/415 nm
Reagent pipetting	Diluent (H_2O)

CO2-L

Bicarbonate Liquid

R1	25 µL	65 µL	
R2	–	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	1 µL	–	–
Decreased	1 µL	–	–
Increased	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: Ammonia/Ethanol /CO_2 Calibrator
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 a primary standard traceable to NIST.

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 6 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.

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a bicarbonate concentration of 22 mmol/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 600 (approximate hemoglobin concentration: 372.6 µmol/L or 600 mg/dL).

Lipemia (Intralipid):[11] No significant interference up to an L index of 1800. 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]

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

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 35 g/L (233.5 µmol/L) (simulated by human immunoglobulin G).

An abnormally elevated concentration of ambient carbon dioxide (CO_2) may occur under certain environmental conditions in the laboratory. The fluctuating ambient CO_2 concentration may interfere with the CO_2-L assay leading to higher CO_2 results. Under these circumstances, the reduction of the re-calibration interval may become necessary if the laboratory is unable to keep the ambient CO_2 concentration at a normal level by appropriate countermeasures.

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

2-50 mmol/L

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2 mmol/L
Limit of Detection	= 2 mmol/L
Limit of Quantitation	= 4 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 bicarbonate samples.

Expected values[1]

22-29 mmol/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.

Repeatability	*Mean mmol/L*	*SD mmol/L*	*CV %*
AEC-N[a)]	19.0	0.140	0.7
AEC-A[b)]	31.9	0.334	1.0
Human serum 1	4.84	0.162	3.3
Human serum 2	17.7	0.148	0.8
Human serum 3	23.3	0.180	0.8
Human serum 4	24.0	0.223	0.9
Human serum 5	46.9	0.370	0.8

Intermediate precision	*Mean mmol/L*	*SD mmol/L*	*CV %*

AEC-N[a)]	18.6	0.363	2.0
AEC-A[b)]	32.3	0.433	1.3
Human serum 1	4.55	0.299	6.6
Human serum 2	17.2	0.439	2.6
Human serum 3	23.3	0.456	2.0
Human serum 4	23.8	0.595	2.5
Human serum 5	46.9	0.549	1.2

a) Ammonia/Ethanol/CO2 Control Normal

b) Ammonia/Ethanol/CO2 Control Abnormal

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

Method comparison

Bicarbonate 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) = 64

Passing/Bablok[15]	Linear regression
y = 1.019x - 0.175 mmol/L	y = 1.024x - 0.267 mmol/L
τ = 0.985	r = 0.999

The sample concentrations were between 2.58 and 48.1 mmol/L.

Bicarbonate 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) = 73

Passing/Bablok[15]	Linear regression
y = 1.000x + 0.500 mmol/L	y = 1.003x + 0.510 mmol/L
τ = 0.956	r = 0.998

The sample concentrations were between 2.20 and 46.8 mmol/L.

References

1 Scott MG, Heusel JW, LeGrys VA, et al. Electrolytes and blood gases, in Tietz NW. Textbook of Clinical Chemistry. 3rd ed. Philadelphia, PA: WB Saunders Co 1999;1065-1066.

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3 Segal MA. A rapid electrotitrimetric method for determining CO2 combining power in plasma or serum. Am J Clin Pathol 1955;25:1212-1216.

4 Wilson W, Jesyk P, Rand R, et al. Use of Vickers discrete analyzer for enzymatic determination of the bicarbonate content of serum. Clin Chem 1973;19(6):640.

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8 Guder WG, da Fonseca-Wollheim F, Heil W, et al. Quality of Diagnostic Samples, in brochure: Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory Medicine, 3rd Ed. 2010.

9 Boyanton BL, Blick KE. Stability studies of Twenty-Four Analytes in Human Plasma and Serum. Clin Chem 2002;48/12:2242-2247.

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11 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

12 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

13 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.

14 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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 dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

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