08057494500V7.0

CO2-L

cobas®

Bicarbonate Liquid

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057494190	08057494500	Bicarbonate Liquid (250 tests)	System-ID 2044 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

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 measurements, performed with this assay in human serum and plasma are used, in combination with pH determination, as an aid in the diagnosis and management of respiratory and metabolic acid-base disorders.

For the regulation of the blood acid/base balance, there are three major buffer systems: the bicarbonate, phosphate, and plasma protein buffer system. The bicarbonate buffer is of major relevance, because being coupled to the respiratory system. Bicarbonate is the second largest fraction of anions in plasma after chloride.[1] In addition to bicarbonate ion (HCO_3^-), the anionic fraction of the bicarbonate buffer system also includes the carbonate ion (CO_3^{2-}), and CO_2 carried as carbamino compounds with plasma proteins, such as hemoglobin. At the physiological pH of blood, the concentration of carbonate is only 1/1000 that of bicarbonate, and the carbamino compounds are present in only low quantities, so that both fractions are generally not mentioned specifically in clinical routine acid/base analysis. Because the dissolved CO_2 fraction (pCO_2; depending on the partial pressure), and carbonate fractions (H_2CO_3) are rather low, the terms bicarbonate and total carbon dioxide are often used interchangeably in clinical chemistry practice.[1] The equilibrium between HCO_3^- with H_2CO_3 thus acts as a buffer pair to minimize changes in blood hydrogen ion (H^+) concentration and thus the pH value. An increase in blood H^+ concentration (i.e., a decrease in blood pH) results in the reduction of plasma bicarbonate levels, whereas a decrease in blood H^+ concentration (increase in blood pH) causes an increase in plasma bicarbonate levels.[2] The interplay between the kidneys and respiratory system ensures the regulation of blood bicarbonate levels and helps maintain the body's acid-base balance.[1,2] The kidneys eliminate acids in the urine and regulate the concentration of bicarbonate in blood.[3] The respiratory system contributes to the regulation of blood bicarbonate levels through expiration and the control of CO_2 levels. When CO_2 levels rise, such as during increased metabolism or exercise, the respiratory system increases the rate and depth of breathing to eliminate excess CO_2.

Clinical conditions characterized by primary disturbances in bicarbonate ion concentrations are classified as metabolic disturbances of acid-base balance, while those characterized by primary disturbances in pCO_2 are classified as respiratory disturbances.[1] Acid-base disorders that are respiratory in nature arise as a result of abnormal CO_2 removal by the lungs, whereas metabolic disorders are caused by aberrant regulation of bicarbonate.[2,3] Disorders that cause an increase of bicarbonate ions (reduction of H^+) or decrease of bicarbonate ions (increase of H^+) are termed alkalosis and acidosis, respectively. Consequently, acid-base disturbances are traditionally classified by their cause as metabolic acidosis, metabolic alkalosis, respiratory acidosis, or respiratory alkalosis.[1]

Low bicarbonate levels have been associated with conditions such as renal diseases, diabetic ketoacidosis, severe diarrhea, and certain drug toxicities. High bicarbonate levels can occur in conditions like prolonged vomiting, certain kidney disorders, and certain diuretic use.[1,2] When CO_2 is abnormally retained by the lungs, e.g. in chronic obstructive pulmonary disease, the kidneys increase the production and reabsorption of bicarbonates to compensate for the respiratory acidosis caused by CO_2 retention. This helps in stabilizing the pH level in the blood.[1] Monitoring the bicarbonate levels in serum or plasma can thus provide valuable information about the body's acid-base status and helps in the diagnosis and management of these imbalances.[4,5]

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.[6,7] These methods are often cumbersome, time-consuming, technique-oriented, and/or require special equipment. Enzymatic procedures using phosphoenolpyruvate carboxylase (PEPC) have been described.[8,9]

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

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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 1 hour.[10] It has been reported that alkalinized serum stored in open cups is stable for up to 4 hours.[10]

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.

Separate from erythrocytes and store tightly stoppered.

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

Stability:	7 days at 4-8 °C[11]
	40 hours at 15-25 °C[12,13]
	Storage of serum at -20 °C or -80 °C for up to 6 months had no significant effect.[14]

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)	505/415 nm		
Reagent pipetting		Diluent (H_2O)	
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 ± 2.2 mmol/L of initial values of samples ≤ 22 mmol/L and within ± 10 % for samples > 22 mmol/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 600 (approximate hemoglobin concentration: 372.6 µmol/L or 600 mg/dL).

Lipemia (Intralipid):[15] 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.[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.

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.

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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[19]

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) and **cobas c** 703 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[20]	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[20]	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.

Bicarbonate 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) = 74

Passing/Bablok[20]	Linear regression
y = 1.010x - 0.422 mmol/L	y = 1.017x - 0.502 mmol/L
τ = 0.965	r = 0.999

The sample concentrations were between 5.19 and 48.3 mmol/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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