BICARBONATE

Diagnostic reagent for determination of Bicarbonate concentration.
Liquid. Monoreagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
A5010	100 mL	HN320	300 mL	MBC20	400 mL	RD2031	225 mL
A5020	500 mL	LBC20	160 mL	M3C20	120 mL	TBC20	400 mL
BB025	160 mL	LM38	160 mL	M4C20	350 mL	8A2034	450 mL
BY2034	400 mL			MDC20	120 mL		

Changes made in the instructions for use are marked as grey.

INTENDED USE

This test is used to determine the quantitative bicarbonate concentration in serum.

GENERAL INFORMATION

Bicarbonate is the second largest fraction of plasma anions (after Cl^-). It is traditionally defined to include (1) the plasma bicarbonate ion (HCO_3^-), (2) the carbonate ion (CO_3^{2-}) and (3) CO_2 bound in plasma carbamino compounds (RCNHCOOH). Actual bicarbonate ion concentration is not measured in clinical laboratories. The analyte usually measured in plasma is total CO_2, which includes bicarbonate and dissolved CO_2 (dCO_2), but is often referred to as "serum bicarbonate". At the pH of the blood, the amount of dissolved CO_2 is 700 to 1000 times greater than the amount of carbonic acid (H_2CO_3); hence $cdCO_2$ is the term used to refer to their combined concentration. It is calculated from the solubility coefficient of CO_2 in blood at 37°C (α=0.0306 mmol/L per mm Hg) multiplied by measured PCO_2 in mm Hg. Therefore, at a PCO_2 of 40 mm Hg, the $cdCO_2$ is 1.224 mmol/L (0.0306 mmol/L × 40 mm Hg). This $cdCO_2$ value can then be used to calculate the total bicarbonate concentration in the Henderson-Hasselbalch equation. This equation can be written as follows:

Equation 1:

$$pH = 6.1 + \log \frac{cHCO_3^-}{cdCO_2}$$

Here $cdCO_2$ (0.0306 mmol/L per mm Hg) is equal to PCO_2 and "6.1" is the pK value for the carbonic acid/bicarbonate system. The average normal ratio of the concentration of bicarbonate (HCO_3^-) and $cdCO_2$ (dissolved CO_2 and H_2CO_3) concentrations in plasma is 25 (mmol/L)/1.25 (mmol/L)=20/1. Therefore, any relative change in the concentration of bicarbonate or dissolved CO_2 must be accompanied by a change in pH. Such changes in this ratio can occur through a change in $cHCO_3^-$ (renal component) or PCO_2 (respiratory component). Clinical conditions described as metabolic disorders of acid-base balance are classified as primary disorders in $cHCO_3^-$. Those described as respiratory disorders are classified as primary disturbances in $cdCO_2$ (PCO_2). Various compensatory mechanisms attempting to restore the normal ratio of $cHCO_3^-/cdCO_2$ may cause changes in the concentration of bicarbonate, dissolved CO_2 or both. The definition of acid-base balance involves the assessment of carbonic (H_2CO_3, HCO_3^-, CO_3^{2-} and CO_2) and non-carbonic acids and conjugated bases in terms of input (intake + metabolic production) and output (excretion + metabolic conversion) over a given period of time. The acid-base status of body fluids is typically assessed by measurements of total CO_2, plasma pH and PCO_2, since the bicarbonate/carbonic acid system is the most important buffering system in mammals. Although PK values (6.1) are far from the normal plasma pH of 7.4, the most important plasma buffer is the bicarbonate/carbonic acid pair. The normal bicarbonate/dCO_2 ratio is 20:1, which is outside the 10:1 or 1:10 ratio at which buffers work best. In fact, the effectiveness of the bicarbonate buffer is based on the fact that the lungs can easily excrete or retain CO_2 and that it is available in higher concentrations than other buffers except hemoglobin. In addition, the renal tubules can increase or decrease the rate at which bicarbonate is recovered from glomerular filtration.[1] The importance of a relatively high bicarbonate concentration (relative to the H^+ ion) becomes apparent when one considers that at normal plasma pH, 5 mmol/L lactate (pK≈3.86) produces ≈5 mmol/L H^+ ions, which is remarkable given that a normal H^+ ion concentration is only 40 mmol/L. The buffer value (β) is defined as the amount of base required to cause a 1 unit change in pH. The buffer value of bicarbonate buffer in plasma is 55.6 mmol/L.[2]

Like any buffer, the bicarbonate/carbonic acid buffer system contains a weak acid (in this case carbonic acid, H_2CO_3) and its conjugated base (bicarbonate ion, HCO_3^-) in dynamic equilibrium as shown in equation 2.

$$H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow CO_2 + H_2O$$

The acidity of a solution is governed by the concentration of hydrogen ions (H^+) present. For instance, if a disease process results in an increase in the concentration of hydrogen ions, one might expect the body to become more acidic. However, the bicarbonate buffer system resists this change because the excess of hydrogen ions drives the reaction in equation 2 to the right.[3]

The bicarbonate content of serum or plasma is an important indicator of electrolyte distribution and anion deficiency. In addition to determining pH, 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. While expressing acid-base state, an arterial

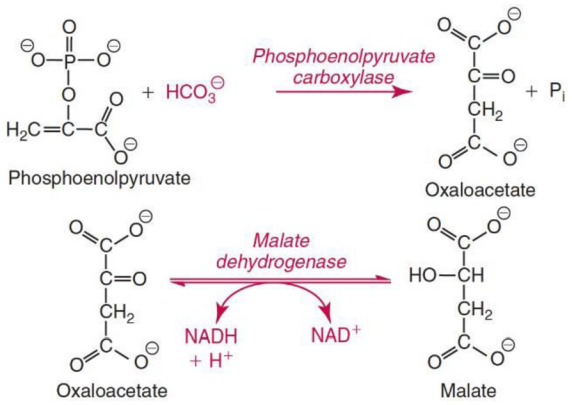
blood pH less than 7.35 is defined as acidemia and an arterial blood pH greater than 7.45 is defined as alkalemia. Acidosis and alkalosis often refer to pathological conditions leading to acidemia or alkalemia. For example, in common acid-base disorders such as lactic acidosis and diabetic ketoacidosis, intermediate organic acids that are normally metabolized to CO_2 and water (lactic acid and β-hydroxybutyric acid, respectively) can accumulate significantly, causing acidemia. In addition, multiple types of pathologic processes can occur simultaneously leading to a mixed acid-base disorder in which blood pH may be low, high or within the reference range.[1] A reduced bicarbonate concentration may mean that the body's main buffer is being used to buffer excess acid (hydrogen ion) production, as in lactic acidosis or ketoacidosis. It may also indicate a problem with bicarbonate loss from the gastrointestinal tract in diseases with diarrhea symptoms. Reduced H^+ excretion seen in renal failure is another reason. Consequently, decreased bicarbonate concentration is the distinguishing feature of metabolic acidosis. Increased bicarbonate concentration may indicate significant losses of acidic fluid, as seen in loss of gastric fluid due to persistent vomiting or prolonged nasogastric aspiration. The use of diuretics may also cause a decrease in bicarbonate concentration due to loss of extracellular fluid. Alternatively, increased bicarbonate concentration may be a chronic adaptation of the kidney to high $PaCO_2$ levels in people with chronic respiratory diseases associated with CO_2 retention. In general, a high bicarbonate concentration indicates the presence of metabolic alkalosis.[3]

TEST PRINCIPLE

Enzymatic method

In this enzymatic method, the sample is first alkalized to convert all CO_2 and carbonic acid to HCO_3^2. The first of the two-step reactions uses phosphoenolpyruvate as substrate. The reaction uses phosphoenolpyruvate kinase (PEPC) as the enzyme. The second step involves the transfer of a hydrogen ion from NADH+H^+ to oxaloacetate catalyzed by malate dehydrogenase (MDH).

The decrease in absorbance at 405 nm resulting from the oxidation of NADH+H^+ is proportional to the concentration of bicarbonate in the sample. The enzymatic reactions are as follows:



REAGENT COMPONENTS

Tris Buffer	PH: 7.5
PEP	≤ 18.5 mmol/l
Sodium Azide	≤ 0.2 %
NADH	≤ 0.9 mmol/l
PEPC	≤ 400 U/L
MDH	≤ 4100 U/L

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[4]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Bicarbonate activity stability in serum and plasma[17]

8 hours +20/+25°C
3 day +2/+8°C
2 week -20°C

Annotation:

- EDTA, citrate and oxalate should not be used as anticoagulants as they can affect the results. Samples should be stored on ice and analyzed within 1 hour. CO_2 can cause erroneous values as it will diffuse out of the sample quickly. Samples should therefore be kept tightly sealed (up to 6mmol).
- Samples must be clean, turbid samples should not be used for hemolysis. Disposable test tubes are used (in photometers also in disposable reaction cuvettes) and the reaction cuvette is washed with 1N HCl solution and then with distilled water.

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an Bicarbonate Calibrator. We recommend:

Bicarbonate Calibrator
Ref.No: ZB84

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 7 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Bicarbonate Control Set
Ref.No: ZB100

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS[5,16]

Serum/Plasma	***mmol/L***
Cord	14 - 22
Newborn	13 - 22
Premature - 1 week	14 - 27
Infant	20 - 28
Child	20 - 28
Adult	22 - 29
> 60 years	23 - 31

Annotation:

- Reference ranges are influenced by physiological changes that occur throughout life.[5] Throughout childhood, from 6 to 12 months old up to the age of 18 years, there is a steady increase in serum HCO_3^2 (by 5 to 7 mmol/L), which is due to a decrease in respiratory rate and subsequent increase in PCO_2.[6] During pregnancy, HCO_3^2 levels decrease by 2 to 3 mmol/L (due to increased respiratory rate caused by the increased concentration of progesterone associated with pregnancy).[5]

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

Reference interval has been verified by using CLSI EP28-A3c protocol.[7]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[8]

The determined analytic measuring interval for Bicarbonate is 3 – 50 mmol/L.

Detection Capability

Limit of Detection (LoD): 1 mmol/L

Limit of Quantitation (LoQ): 3 mmol/L

LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[9]

Linearity

This method shows measurement linearity in the activities up to 50 mmol/L.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[10]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[11]

Repeatability (Within Run) SD (standard deviation) and CV% values of Bicarbonate have been given in the table 1 and 2 respectively.

Table 1. Bicarbonate Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
15.1 mmol/L	0.3	1.98	80
26.8 mmol/L	0.59	2.20	80

Annotation:

- This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[12]

Table 2. Bicarbonate Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
15.1 mmol/L	0.4	2.64	80
26.8 mmol/L	0.65	2.42	80

Annotation:

- This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[12]

Interference

Endogenous interferant and analyte concentrations that have been used in the Bicarbonate scanning tests has been

determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[13,14]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Bicarbonate interference scanning test is appropriate, is determined as ±25%.[15]

In Bicarbonate test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Hemoglobin	: ≤ 800 mg/dL
Bilirubin	: ≤ 50 mg/dL
Intralipid	: ≤ 1000 formadine
Ascorbic Acid	: ≤ 50 mg/dL

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[14]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.
CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

REFERENCES

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests