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BICARBONATE Enzymatic Method

Reagent for quantitative determination of total Carbon dioxide (CO_2) in human serum or plasma.

REF 99832	**R1** 8 x 30 mL	**R2** 1 x 30 mL
REF 99852	**R1** 6 x 100 mL	**R2** 1 x 30 mL

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)

Total carbon dioxide content of plasma consists of CO_2 dissolved in an aqueous solution, CO_2 loosely bound to amine groups in proteins (carbamino compounds), bicarbonate HCO_3^-, vanishingly small amounts of CO_3^{2-} ions and carbonic acid (H_2CO_3). Approximately 90 % of total carbon dioxide in plasma is in form of bicarbonate.
Measurement of total CO_2 as a part of an electrolyte profil (Na^+, K^+, Cl^-), and with blood gases and pH values, is useful chiefly to evaluate HCO_3^- concentration in assessment of acid-base disorders resulting from metabolic or respiratory causes.

PRINCIPLE (4) (5)

Enzymatic methods for determining total CO_2 as bicarbonate and dissolved gases. Reaction scheme is as follows:

$$HCO_3^- + \text{Phosphoenopyruvate} \xrightarrow{\text{PEPC}} \text{Oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + NADH \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

The decrease in absorbance due to the oxidation of NADH in NAD^+ is directly proportional to the amount of total CO_2 in the specimen and is measured at 380 nm.

REAGENTS COMPOSITION

Vial R1 **WORKING REAGENT**

Phoshoenolpyruvate	8.0	mmol/L
NADH	1.6	mmol/L
PEPC (Phosphoenolpyruvate carboxilase)	> 1000	IU/L
MDH (Malate dehydrogenase)	> 200	IU /L
Buffer pH (20°C) 8.0 ± 0.1	66	mmol/L
Stabiliser		

Before reconstitution: Xn, R22: Harmful if swallowed
S22: Do not breathe dust
S28: after contact with skin, wash immediately with plenty of water
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Once reconstituted: None

Vial R2 **STANDARD**

Sodium bicarbonate	27	mmol/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Add promptly to the contents of vial R1 the amount of distilled or deionised water stated on the label.
Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes).
To avoid CO_2 contamination, use fresh distilled water, not stored longer than 1 day. Do not pipette by mouth.

STABILITY AND STORAGE

Store away from light, well capped in the original vial at 2-8°C.

- Reagent (vial R1) and Standard (vial R2): Transfer requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 4 months when free from contamination. **Recap promptly after use.**
- Discard any reagent if cloudy or if absorbance measured at 380 nm is < 1.000.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2) (6)

Plasma or Serum, venous. Collect specimen anaerobically. Heparin is the preferred anticoagulant.

Do not store specimen more than 1 hour at 2-8°C.

INTERFERENCES (3)

Studies on sera with Cobas Mira (**340 nm**) show results as follows:

Interferent	Bicarbonates in specimen (mmol/L)	Results
Ascorbic acid	27,9 mmol/L	No interference up to 25 mg/dL
Total Bilirubin	31,3 mmol/L	No interference up to 500 µmol/L
Haemoglobin	25,6 mmol/L	No interference up to 248 µmol/L
Glucose	28,6 mmol/L	No interference up to 1000 mg/dL
Lipemia	26,3 mmol/L	No interference of the turbidity up to 0.283 abs (measured at 600 nm)

Studies on sera with Spectrophotometer (**380 nm**) show results as follows:

Interferent	Bicarbonates in specimen (mmol/L)	Results
Ascorbic acid	17,2 mmol/L	No interference up to 25 mg/dL
Total Bilirubin	24.1 mmol/L	No interference up to 500 µmol/L
Haemoglobin	24,2 mmol/L	Positive interference above 130 µmol/L
Glucose	23,0 mmol/L	No interference up to 1000 mg/dL
Lipemia	13,6 mmol/L	Interference above 0.050 abs (measured at 600 nm)

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.
3. Demineralised water for the preparation of reagent.

CALIBRATION

- Standard (vial R2) enclosed in the kit measured in standardized conditions with enzymatic method and aqueous standard traceable to NERL Standard
- or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases:

1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- Normal Control Ethanol Ammonia Bicarbonate REF 95013
- Pathological Control Ethanol Ammonia Bicarbonate REF 95023
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

Carbon dioxide total	(mEq/L)	(mmol/L)
Adult:	23-29	[23-29]
> 60 years:	23-31	[23-31]
> 90 years:	20-29	[20-29]

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCES CHARACTERISTICS (7)

Within run n = 20	***Low level***	***High level***
Mean mEq/L	10.0	40
S.D. mEq/L	0.7	0.6
C.V. %	7.3	1.4

Between run n = 20	***Low level***	***High level***
Mean mEq/L	10.0	40.0
S.D. mEq/L	0.8	1.6
C.V. %	8.0	3.9

Detection limit: approximately 3.0 mEq/L

Sensitivity for 1 mEql/L: approximately 0.010 ΔAbs.

Comparison study with a commercially available reagent (enzymatic method): 67 sera within 15 and 43 mmol/L have been evaluated with both reagents. Results compared by least squares regression are as follows:

y = 0,96 x + 0,40 r = 0,96

X (mmol/L)	Acceptable Inaccuracy (4)	Y calculated value	Observed Inaccuracy	Conclusion
25.00	+/-3.6	24.4	0.6	Passed
40.00	+/-4.0	38.8	1.2	Passed
55.0	+/-4.2	53.2	1.8	Passed

LINEARITY

The assay is linear up to 50 mEq/L (50 mmol/L).

Above, dilute specimen with demineralised (CO_2-free) water and reassay taking into account dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Pipette in test tubes at 37°C (30°C):	**Blank**	**Standard**	**Assay**
Working Reagent (vial R1)	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL
Mix well. Incubate for 5 minutes at 37°C (30°C). Use a 1 cm path length cuvette and read absorbance at 380 nm (*) against demineralised water**.**			

Notes:

Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support

(*) May be used at 340 nm, dilute reagent (1+3) with fresh demineralised water , prepare and use extemporaneously

CALCULATION

Calculate the result as follows:

$$\text{Total } CO_2 {}^{(*)} = \frac{(\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Assay}})}{(\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Standard}})} \times \text{Standard concentration}$$

(*) Total CO_2 is approximately 10 % higher than Bicarbonate.

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1104-1124.*

(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 166-167, p.214-215*

(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-84 to 3-88*

(4) *NORRIS KA, ATKINSON AR, SMITH WG. CLIN. CHEM. (1975) 21: 1093.*

(5) *Forrester RL, Wataji JJ, Silverman DA, Pierre JK Clin. chem. (1976) 22: p.243-245*

(6) *Henry RJ, Clin. chem. « Principles and technics » Harper and Row New-York (1974)*

(7) *National Comittee for clinical laboratory standards. User evaluation of precision performance of clinical chemistry devices. NCCLS (1984), NCCLS Publication EP5-T*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with