MEDICA

IVD CE

REF 10209-4 4 x 23 mL

CARBON DIOXIDE (CO_2)

Wedges each contain a usable volume of 23 mL of reagent.

INTENDED USE

The EasyRA carbon dioxide reagent is intended for the quantitative determination of Carbon Dioxide (CO_2) in human serum and plasma, using the MEDICA "EasyRA Chemistry Analyzer" in clinical laboratories. Bicarbonate/carbon dioxide measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance.
For *in-vitro* diagnostic use only.

SUMMARY AND EXPLANATION

The Carbon Dioxide (CO_2) test is performed to assess respiratory metabolism. The bicarbonate concentration in serum can be used as a screening parameter of non-respiratory acid-base disturbance.

PRINCIPLE OF THE PROCEDURE

This enzymatic method is readily automated. The phosphoenolpyruvate carboxylase (PEPC), which catalyzes the first reaction, is specific for bicarbonate ion (HCO_3^-) and generates oxaloacetate and phosphate. PECP, by disturbing the equilibrium between CO_2 and HCO_3^- drives the conversion of all of the CO_2 to bicarbonate.

$$\text{(I)}\quad \text{Phosphoenolpyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{oxaloacetate} + H_2PO_4^-$$

In the presence of MDH in this second reaction (II), oxaloacetate oxidizes the reduced cofactor. The decrease in concentration of reduced cofactor is monitored at 405 nm and is proportional to the total carbon dioxide in the sample.

$$\text{(II)}\quad \text{Oxaloacetate} + \text{NADH-analog} + H^+ \xrightarrow{\text{MDH}} \text{malate} + NAD^+$$

REAGENTS

Phosphoenolpyruvate (PEP)	≥ 5 mmol/L
PEPC (microbial)	≤ 2000 U/L
Malate dehydrogenase(EC1.1.1.37)	>1000 U/L
NADH analog[1]	≥ 0.30 mmol/L

Non-reactive ingredients: Buffers and stabilizers

Precautions

1. Specimens in sample cups must be analyzed immediately to prevent excessive CO_2 loss.
2. Good laboratory safety practices should be followed when handling any laboratory reagent. (*NCCLS,* GP17-A2).
3. The reagent contains less than 0.1% of sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
4. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
5. Do not use washed cuvettes.

Instructions for Reagent Handling, Storage and Stability

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2º-8ºC. Uncap the reagent only during the performance of a test. Keep the reagent tightly closed when not in use. When used in this way, the reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days programmed on the RFID chip on the reagent wedge. If the analyzer does not have the refrigeration option, the reagents need to be recapped and stored at 2º-8º C. after use. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

SPECIMEN COLLECTION AND STORAGE / STABILITY

Clear unhemolyzed serum and plasma should be used. Lithium Heparin coated tubes may be used for plasma collection. Centrifuge and remove the serum within 2 hours of collection. The specimen should be stored tightly sealed to prevent the loss of carbon dioxide. Minimize exposure of the sample to air Separated serum or plasma should not remain at room temperature (+15°C to +30°C) for longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored refrigerated (+2°C to +8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen (-15°C to -20°C). Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed. [2]

PROCEDURE

Materials Provided
Medica CO_2 Reagent Wedge, REF 10209
Additional materials required
Medica EasyCal CO_2, REF 10654
Medica EasyQC Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area. The on-board stability (10 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check the inside of the neck of the wedge for foam after removing the cap and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test.

Calibration
Medica EasyCal CO_2, REF 10654 is recommended for the calibration of the assay. The calibration interval (10 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control
It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay at least once every 8 hours and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should also follow local, state, and federal quality control guidelines when using quality control materials.

Results

After completion of the assay, the Medica EasyRA chemistry analyzer calculates the CO_2 concentration from the ratio of the change in the corrected unknown sample's absorbance to the change in the corrected absorbance of the calibrator multiplied by the calibrator value.

$$CO_2 \text{ (mmol/L)} = \frac{\Delta A_{U_{405}} - \Delta A_{Blk_{405}}}{\Delta A_{C_{405}} - \Delta A_{Blk_{405}}} \times \text{CalValue}$$

Where A_U and A_C are the absorbance values of unknown sample and calibrator, respectively, A_{Blk} is absorbance of the reagent blank, and "Cal Value" is the concentration of CO_2 in the calibrator (mmol/L).

Expected Values[2]
The reference range for CO_2 in serum is as follows:

Normal: 23-34 mmol/L

These values are suggested guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g., if sample is above assay range)
Avoid using hemolyzed serum or plasma samples..

The Medica EasyRA Chemistry Analyzer flags any result above 45 mmol/L as Linearity High "LH". ". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will effectively extend the reportable range of the CO_2 test to 90 mmol/L.

PERFORMANCE CHARACTERISTICS [5]

Reportable Range
The reportable range is 2.3 to 45.0 mmol/L. Extended range is 2.3 to 90 mmol/L when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (NCCLS, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for CO_2 (y) on the Medica EasyRA Chemistry Analyzer to the performance of a similar CO_2 reagent (x) on the Roche COBAS MIRA Analyzer. The data shown below represents

single determinations on the Medica EasyRA Chemistry Analyzer vs. the average of 2 replicate values obtained on the Roche COBAS MIRA Analyzer.

Number of samples	60	Range of Samples	2.3 to 44.1 mmol/L
Slope	0.9414	y Intercept	0.6015
Correlation Coefficient	0.9921	Regression Equation:	Y = 0.9414*X + 0.6015

The following table lists the data obtained in a comparison of matched serum (x) and Li-Heparinized plasma (y) samples using the Medica Reagent for CO_2 on the Medica EasyRA Chemistry Analyzer. The data below represents a single plasma determination vs. the average of two replicate serum values.

Number of Samples	75	Range of Samples	3.8 to 41.3 mg/dL
Slope	1.032	y Intercept	-0.012
Correlation	0.972	Regression Equation	Y = 1.03*X – 0.012

Imprecision (NCCLS, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level mmol/L	Within Run SD mmol/L	Within Run CV %
32.2	0.35	1.09
27.8	0.48	1.73
18.3	0.30	1.63

Total Imprecision:

QC Level mmol/L	Total Imprecision SD mmol/L	Total Imprecision CV %
32.2	1.71	5.30
27.8	1.49	5.34
18.3	0.93	5.05

Linearity (NCCLS, EP6-A)
Linear from 2.3 to 45.0 mmol/L, based on the linear regression Y = 0.9861*X + 0.2022.

Limit of Blank (LOB):	0.56 mmol/L	(NCCLS, EP17-A)
Limit of Detection (LOD):	1.00 mmol/L	(NCCLS, EP17-A)

Interfering Substances (NCCLS, EP7-A)
Less than 10% interference was classified as “no significant interference”.
There is significant interference at hemoglobin levels above 125 mg/dL. Avoid using hemolyzed serum samples.
No significant interference was found with levels up to 20 mg/dL of bilirubin.
No significant interference was found with levels up to 850 mg/dL of triglycerides (using Intralipid*).

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[3, 4]

REFERENCES

1 U.S. Patent No. 5,801,006.
2 Clinical Laboratory Standards Institute (CLSI), Procedures for the Handling and Processing of Blood Specimens, Approved Guideline, CLSI publication G44-A4, Wayne, PA (2010).
3 Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
4 Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.
5 Data on file at Medica.

EasyRA Assay Parameters (CO_2)

Wavelength (nm)	405
Reaction Type	Special End Point (3)
Reaction Direction	Decrease
Reagent Blank	Yes (with each calibration)
Sample Blank	No
Reaction Time	6.0 min
Calibration interval (maximum)	10 days
Reagent on-board stability	10 days

Serum

Sample volume (µl)	3.0
Diluent volume (µl)	0
Reagent volume (µl)	270
Decimal Places (default values)	1
Units (default values)	mmol/L
Dilution Factor to extend measuring range	1:1
Linearity	2.3 to 45.0 mmol/L

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