

REF 77475

Intended Use

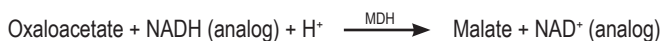
Eon Bicarbonate Reagent (Total CO₂) is for the quantitative determination of bicarbonate in serum and plasma using the Eon 100 Analyzer.

Summary ^{1,2}

Bicarbonate is the second most common anion present in plasma and not only includes bicarbonate but also carbonate ions and carbamino compounds. At normal physiological pH, the ratio of bicarbonate to the other two compounds is approximately 20 to 1.

Serum or plasma bicarbonate concentration is typically measured as part of an electrolyte panel and is an index of Hydrogen ion homeostasis (acid-base balance). Changes in the concentration of bicarbonate are typically the result of disturbances in acid-base metabolism, due to underlying respiratory or metabolic disease.

The Eon Bicarbonate Reagent is an enzymatic rate method based on the following reaction sequence:



The rate of production of NAD⁺ analog produces a decrease in absorbance at 405nm, which is directly proportional to the concentration of bicarbonate present in the sample or control material being assayed.

Where

PEP = Phosphoenolpyruvate

PEPC = Phosphoenolpyruvate Carboxylase

MDH = Malate Dehydrogenase.

Reagents

Composition

Eon Bicarbonate **REAG** contains PEPC (microbial) ≥ 150 U/L; PEP ≥ 8 mmol/L; NADH analog, ≥ 0.5 mmol/L; MDH (porcine) ≥ 3000 U/L as well as buffer, stabilizers and preservatives.

Warnings and Precautions

For *in vitro* diagnostic use.

Handle and dispose of all human source materials as though capable of transmitting infectious agents using the universal precautions³ recommended by the Centers for Disease Control and Prevention (CDC). Do not pipette by mouth; do not eat, drink, smoke or apply cosmetics in areas where specimens are handled. Clean up spills immediately with a 0.5% sodium hypochlorite solution.

This product contains sodium azide, which can react with both copper and lead in drains to form explosive compounds. Flush drain with copious amounts of water after disposal.

Preparation

Eon Bicarbonate **REAG** is ready for use on the Eon 100 Analyzer as packaged.

Storage and Stability

Store the reagent at 2-8°C. Unopened reagents are stable to the expiration date on the bottle label. The reagent is stable for 14 days onboard the Eon 100 Analyzer.

Specimens

Serum and Plasma Collection and Storage

Serum or plasma (lithium heparin) is the preferred specimen. Do not analyze whole blood. Collect specimens by venipuncture according to accepted clinical protocol. Separate the serum or plasma sample from the cells as soon as possible.

For the most accurate results, assay the specimen as soon as possible after opening the collection tube. Specimens may be stored for up to 16 hours at 25°C⁴ if the collection tube is tightly capped.

Procedure

Materials Provided

The Eon Bicarbonate Reagent kit includes the following components:

4 x 24mL bottles of Eon Bicarbonate **REAG**

Materials Required But Not Provided

Eon Calibrator **REF** 77111

Eon Serum Control **REF** 77131

Reagent Installation and Use

Refer to the user manual⁵ for additional information on installing reagents, programming the analyzer and running samples, calibrators, and controls. Do not remove the caps from the reagent bottles until you are ready to install the reagent on the Eon 100. Before installing, mix the reagent by gently inverting the reagent bottle several times. Record the date opened on the label and insert the reagent bottle into the designated position on the reagent tray. Let the reagent equilibrate on the instrument for at least 30 minutes before use.

Calibration

Calibrate the instrument after loading new reagent, after maintenance and whenever quality control results fall outside established limits. Under typical conditions, calibration is stable for 7 days. Refer to the user manual⁵ for calibration procedures.

Quality Control

Assay at least two levels of serum control daily in accordance with an accepted quality control program. Control materials may be of human or animal origin, but should represent both clinically normal and abnormal levels of bicarbonate. Controls should also be assayed after calibrating, maintaining the instrument and after loading a new reagent. Controls may be assayed more frequently based on laboratory workflow and the discretion of the user.

Calculations

All calculations are performed by the Eon 100.

Limitations

Interfering Substances

Studies to determine the level of interference from biological compounds that may be normally present in serum or plasma were carried out on the Eon 100. No significant interference (± 10%) in bicarbonate recovery was observed in the presence of:

Lipemia (Intralipid® measured as Triglycerides) up to 1000 mg/dL

Bilirubin up to 60 mg/dL (1026 μmol/L)

Hemoglobin up to 1000 mg/dL (10 g/L)

Triglycerides up to 500 mg/dL (5.65 mmol/L)

Many other substances can affect bicarbonate results. For additional information, refer to *Effects of Drugs on Clinical Laboratory Tests*⁶ and *Effects of Preanalytical Variables on Clinical Laboratory Tests*⁷.

The results of this assay should only be interpreted in conjunction with other diagnostic test results, clinical findings and the patient's medical history.

Limits and Ranges

Measuring Range

The Measuring Range of the assay defines the acceptable upper and lower limits of the range within which values may be reported. It is comprised of the Limit of Detection (lower limit) and the highest measured point that will be achieved throughout the lifespan of the reagent (upper limit).

Serum / Plasma: 3 - 45 mmol/L

Limit of Blank and Limit of Detection

The Limit of Blank (LoB) is the highest result observed in a sample that does not contain the analyte being measured. The LoB for this reagent was determined as per CLSI EP17-A.

Eon Bicarbonate Reagent LoB = 1 mmol/L

The Limit of Detection (LoD) is the lowest concentration of analyte in a sample which can be detected and is calculated using the following formula (CLSI EP17-A)

$$\text{LoD} = \text{LoB} + 1.645 (\text{SD}_{\text{low concentration sample}})$$

Eon Bicarbonate Reagent LoD = 3 mmol/L

Sensitivity

A change in absorbance of 0.0069 on the Eon 100 corresponds to a bicarbonate concentration of approximately 1.0 mmol/L.

Expected Values²

Venous Plasma/Serum: 23 - 29 mmol/L

The quoted range should serve as a guide only. It is recommended that each laboratory verifies this range or establishes a reference interval for the population that it services.⁸

Performance Data

Precision

Imprecision (Repeatability) was determined in accordance with CLSI EP5-A2 guidelines. Three levels of serum were analyzed in duplicate, two times per day, over a period of twenty days (40 runs).

A summary of the results are shown in the table below.

		Level 1	Level 2	Level 3
Number of Data Points		80	80	80
Mean (mmol/L)		12	21	31
Within Run	SD	0.7	0.7	1.1
	CV %	6.0	3.5	3.5
Total	SD	1.2	1.6	2.0
	CV %	10.3	7.5	6.5

Method Comparison

A comparison of the Eon Bicarbonate assay (y) with a commercially available method (x) was performed based on CLSI EP9-A2 guidelines. A total of 202 human serum samples were assayed in the range from 4 to 45 mmol/L.

The following statistics were obtained:

Linear Regression
 $y = 0.990x - 0.651$
 $R = 0.9795$


Deming Regression
 $y = 1.011x - 1.101$
 $R = 0.9795$

References

1. Zilva JF, Pannall PR. Clinical chemistry in diagnosis and treatment. 3rd Ed. London: Lloyd-Luke; 1979.
2. Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 4th Ed. Philadelphia PA: W.B. Saunders Company; 2006.
3. Department of Health and Human Services (US) [HHS] Recommendations for Prevention of HIV Transmission in Health Care Settings. MMWR 36(SU02); 001 1987, August 21. Available at: <http://www.cdc.gov/mmwr/preview/mmwrhtml/00023587.htm> (Accessed 30 March 2010).
4. Boyanton BL, Blick KE. Stability studies of twenty four analytes in human plasma and serum. Clin Chem 2002; 48:2242-7.
5. Eon 100 User Manual (L7283) Vital Diagnostics, Lincoln, RI.
6. Young DS. Effects of drugs on clinical laboratory tests. 5th Ed. Washington DC: AACC Press; 2000.
7. Young DS. Effects of preanalytical variables on clinical laboratory tests. 3rd Ed. Washington DC: AACC Press; 2007.
8. Wachtel M, Paulson R, Plese C. Creation and verification of reference intervals. Lab Med 1995; 26: 593-7.














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GLOSSARY OF SYMBOLS

	Manufacturer		Batch code / Lot number		In vitro diagnostic medical device
	Contents		Caution		Consult instructions for use
	Catalog number		Temperature limitation		Use by / Expiration date
	Date opened		Number of days onboard stability		Authorized Representative in the European Community
	Reagent				