HORIBA Instruments Incorporated
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# Pointe Carbon Dioxide Reagent Set

## Intended Use
For the quantitative determination of Carbon Dioxide in serum using the Yumizen C560 analyzer. For *in vitro* diagnostic use only. **Rx Only.**

## Method History
Early methods for the determination of carbon dioxide were based on either volumetric or manometric determination of the $CO_2$ released from a sample by acid treatment. These methods used the instruments of Van Slyke [1,2] until they were replaced by the Natelson microgasometer,[3] which still uses manometric determination of total $CO_2$.
Methods have been developed for Auto Analyzers[4] but these suffer from baseline drift[5] and require equipment which many laboratories do not have. Enzymatic methods for $CO_2$ have been introduced by Wilson,[6] Menson[7] and Norris[8] using phosphoenolpyruvate carboxylase. The present procedure is a enzymatic assay utilizing Phosphoenolpyruvate Carboxylase (PEPC) and a NADH analog.

## Principle
$$PEP + HCO_3^- \xrightarrow{PEPC + Mg^{++}} Oxaloacetate + H_2PO_4^-$$

$$Oxaloacetate + reduced\ cofactor + H^+ \xrightarrow{MDH} Malate + cofactor$$

Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEPC), to form oxaloacetate. The cofactor then in the presence of malate dehydrogenase (MDH) is oxidized by the oxaloacetate. The decrease in absorbance monitored between 405 and 415 nm resulting is proportional to the amount of $CO_2$ in the sample.

## Clinical Significance[5]
The measurement of Carbon Dioxide is useful in the assessment of acid-base balance disturbances. Elevated $CO_2$ is observed in metabolic alkalosis and compensated respiratory acidosis. Low $CO_2$ is observed in compensated respiratory alkalosis and metabolic acidosis. Differentiation between the metabolic and respiratory conditions is only possible through additional laboratory determinations.

## Reagents
$CO_2$ reagent: PEP 6mM, Magnesium Ions 10mM, NADH analog, MDH (porcine) ≥ 1200U/L, PEPC (microbial) ≥ 200U/L, Buffer, pH 7.4 ± 0.1 non-reactive stabilizers with surfactants and preservative.

## Reagent Preparation
Reagent provided as a ready to use liquid.

## Reagent Storage and Stability
Reagent is stable until expiration date indicated on vial label when stored tightly capped at 2-8˚C. (15 months from date of manufacture).

## Reagent Deterioration
1. Reagent should appear clear and pale yellow in color.
2. Do not use if reagent appears to be turbid, this would indicate deterioration.

## Precautions and Hazards
1. Reagents are for *in vitro* diagnostic use only.
2. Do not ingest. Toxicity has not been established.
3. Do not pipet by mouth to avoid $CO_2$ contamination from the expired air.

**Hazards:**
Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture
**Refer to the Safety Data Sheet for this product (SDS-C7502) available by calling 1-734-487-8300**

## Specimen Collection and Storage
1. Fresh, unhemolyzed serum collected under anaerobic conditions is the recommended specimen.
2. The sample may be stored in ice water under anaerobic conditions for up to one hour.[9]

## Interferences
1. Interferences were evaluated for this carbon dioxide method on a Yumizen C560 analyzer. No interference was observed by bilirubin up to 20.0 mg/dl, hemoglobin up to 219.9 mg/dl and lipemia (intralipid) up to 1000 mg/dl. (Using a criteria of >10% variance from control. $CO_2$ level was 26, 23, 25 mmol/L respectively)
2. $CO_2$ from air or the breath of the analyst is a major interference in this assay. Reagent handling, specimen collection, and all storage instructions must be strictly followed to minimize this interference.
3. A number of conditions and substances have been reported to affect serum Carbon Dioxide levels. [10,11,12]

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## Materials Provided
Carbon Dioxide Reagent

## Materials Required but not Provided
1. Yumizen C560 Analyzer
2. Yumizen C560 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

## Limitations
1. Samples exceeding 40 mmol/L must be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. Carbon Dioxide contamination must be avoided. Keep reagent tightly capped when not in use.

## Calibration
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 1 day.

## Quality Control
To monitor the reliability of results, two levels of control sera with known Carbon Dioxide values should be run with patient samples. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

## Expected Values [9]
23-34 mmol/L
It is strongly recommended that each laboratory determine its own reference range.

## Performance
1. Assay Range: 2 - 40 mmol/L
2. Comparison: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

| Method | Carbon Dioxide |
|---|---|
| N | 97 |
| Mean CO2 (mmol/L) | 22.5 |
| Range (mmol/L) | 4-39 |
| Standard Deviation | 6.4 |
| Regression Analysis | y = 0.962x – 2.2 |
| Correlation Coefficient | 0.9543 |

3. Precision: Within Day precision was investigated by running two samples in replicates of 20 on the same day. Day to Day results were obtained by performing one run per day over a span of 20 days. Precision studies were performed using the Yumizen C560 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[13]

Within Day

| Sample | LOW | MID | HIGH |
|---|---|---|---|
| N | 20 | 20 | 20 |
| Mean | 7.8 | 23.9 | 30.9 |
| Standard Deviation | 0.4 | 0.5 | 0.6 |
| Coefficient of Variation (%) | 5.7% | 2.1% | 2.0% |

Total

| Sample | LOW | MID | HIGH |
|---|---|---|---|
| N | 40 | 40 | 40 |
| Mean | 10.0 | 22.6 | 29.4 |
| Standard Deviation | 1.1 | 2.0 | 1.0 |
| Coefficient of Variation (%) | 10.5% | 8.7% | 3.3% |

4. Sensitivity: 2SD limit of detection (95% Conf) = 1 mmol/L

## References

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4. Skeggs, L.T. Jr., Am. J. Clin. Path. 33:181 (1960).
5. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA., pp 884-887 (1982).
6. Wilson, W., et al, Clin. Chem. 19:640 (1973).
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8. Norris, K.A., et al, Clin. Chem. 21:1093 (1975).
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10. Young, D.S., et al, Clin. Chem. 21:1D (1975).
11. Martin, E.W., In Hazard of Medication (Alexander, S.F., Farage, D.J., and Hassan, W.E., Jr. eds.), J.B. Lippincott Co., Philadelphia, PA., and Toronto, Canada, p. 169 (1971).
12. Constantino, N.V., and Kabat, H.F., Am. J. Hosp. Pharm. 30:24 (1973).
13. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992)

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# Pointe Carbon Dioxide Reagent Set

## CHEMISTRY PARAMETERS

| | | | | | |
|---|---|---|---|---|---|
| Chem: | CO2 | No.: | 208 | Sample Type: | Serum |
| Chemistry: | Carbon Dioxide | | | Print Name: | CO2 |
| Reaction Type: | Fixed Time | | | Reaction Direction: | Negative |
| Pri Wave: | 412 | | | Sec Wave: | 505 |
| Unit: | mmol/L | | | Decimal | 0 |
| Blank Time: | 10 12 | | | Reaction Time: | 18 41 |

| | Sample Vol. | Aspirated | Diluent | Reagent Vol. | Diluent |
|---|---|---|---|---|---|
| Standard: | 1.5 ul | --- ul | --- ul | R1: 150 ul | --- ul |
| Decreased: | --- ul | --- ul | --- ul | R2: --- ul | -- ul |
| Increased: | --- ul | --- ul | --- ul | R3: --- ul | -- ul |
| | ☐ Sample Blank | ☑ Auto Rerun | | R4: --- ul | --- ul |

**Slope/Offset Adjustment**

Slope: 1 Offset: 0

| | | | |
|---|---|---|---|
| Linearity Range (Standard) | 2 | 40 | Linearity Limit: |
| Linearity Range (Decreased) | ____ | ____ | Substrate Depletion: |
| Linearity Range (Increased) | ____ | ____ | Mixed Blank Abs: |
| R1 Blank Abs: | ____ | ____ | Uncapping Time |
| Blank Response: | ____ | ____ | Reagent Alarm Limit: |
| Twin Chemistry: | | | ☐ Enzyme Linear Extension |

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

# Pointe Carbon Dioxide Reagent Set

## CALIBRATION PARAMETERS

**Calibrator Definition**

| | | | |
|---|---|---|---|
| Calibrator: | * | Lot No.: | * |
| Exp Date: | * | | |

| **Carousel** | **Pos** |
|---|---|
| Sample Carousel 1 | * |
| Sample Carousel 2 | |
| Sample Carousel 3 | |

**Reagent/Calibration**

| Calibrator | Pos | Lot No | Exp Date | Chem | Conc | Unit |
|---|---|---|---|---|---|---|
| Water | W | * | * | CO2 | 0 | mmol/L |
| Chemistry Calibrator | * | * | * | CO2 | * | mmol/L |

**Calibration Setup**

Chem: CO2

Calibration Settings

| | | | |
|---|---|---|---|
| Math Model: | Two-Point Linear | | |
| Factor: | | Replicates: | 2 |

Acceptance Limits

| | | | |
|---|---|---|---|
| Cal Time: | 24 | Hour | |
| Slope Diff: | --- | SD: | --- |
| Sensitivity : | --- | Repeatability: | --- |
| Deter Coeff: | --- | | |

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-C7502-160

Manufactured by
HORIBA Instruments Incorporated-Pointe Brand
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2°C 8°C IVD

### Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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5449 Research Drive, Canton, MI 48188

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CE

**Symbol Key**

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only

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