June 1974

ADVISORY STATEMENT[a]:

Quick Method for Estimating CO_2 in Cucumber Brines

Commercial cucumber briners are actively interested in evaluating the "controlled fermentation process" recently developed by our laboratory (1). The process includes use of nitrogen for purging of the brine to remove dissolved CO_2 and thereby prevent bloater formation. We devised a method for determining CO_2 in brines (2), but results from the analysis cannot be obtained until the next day. Although our method is highly suitable for use in certain types of brining research, it is desirable to learn the results immediately in some instances, particularly when one is testing the efficiency of CO_2 removal from brine by the purging system.

The Harleco CO_2 Apparatus (3) was designed for rapid (about one minute) determination of CO_2 in blood. By careful handling of the sample, this simple apparatus can be used to determine CO_2 in cucumber brines. It is important to recognize, however, that blood has a pH of about 7.0, while that of fermenting cucumber brines varies between about 5.8 and 3.1. CO_2 is trapped in the bicarbonate form in blood and is not volatile; but, at pH values below 6, as with brines, the CO_2 is highly volatile and easily lost. Extra care must be taken in sampling and analyzing for CO_2 under these conditions. The following procedure is recommended:

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[a]Prepared by H. P. Fleming, R. L. Thompson and T. A. Bell of the Food Fermentation Laboratory, Southern Region, Agricultural Research Service, U.S. Department of Agriculture; and Department of Food Science, North Carolina State University, Raleigh, 27607.

Sampling Procedure:

Samples may be taken from brining tanks through the tank sampling slot by means of the syringe device described previously (2).

Alternatively, samples may be taken through a siphoning arrangement as described herein. "A suitable length of 3/16 inch diameter stainless steel tubing (sealed at one end with lead or solder and perforated with several 1/16 inch diameter holes for a distance of 6 to 8 inches from the sealed end) is inserted through an opening in the false head, down into the brine toward the center of the vegetable material," (quoted from Etchells and Jones, 1946, ref. no. 4). A rubber tube is connected to the end of the steel tubing extending above the brine surface. The other end of the tube is fitted to a glass "T" connector which, in turn, is connected to another piece of rubber tubing of sufficient length to extend over the side of the tank and below the brine surface. The steel tube can be raised or lowered in the tank to take samples from various depths. The "T" is capped on the open side with an appropriate size rubber serum stopper. To obtain a sample, siphoning action is begun. After allowing brine from the tank to flush out the tubing, an 8.5 ml sample is taken through the rubber septum of the "T" by means of a 12-ml plastic, disposable syringe (20 gauge needle). The brine sample is drawn slowly to prevent loss of CO_2 from solution and is injected immediately into a Vacutainer tube (10 ml draw, Becton-Dickinson, ref. no. 5). These tubes contain 0.5 ml of about 3 N NaOH, which must be added to them prior to adding the brine sample. Important: the flow of brine through the glass "T" will not contain bubbles unless

there is an air leak in the system; avoid this! The sampling arrangement is shown in Figure 1.

Analysis:

Samples contained in the Vacutainers should be taken into the quality control laboratory and allowed to equilibrate to the same temperature as the standard prior to analysis. Follow the procedure outlined in the brochure which accompanies the Harleco Apparatus for the analysis. The procedure calls for determining a standard with each set of samples. It is important that the temperature of the sample and the environment in which it is analyzed be the same as for the standard.

Calculations:

The Harleco instructions for calculation result in concentrations being expressed as millimoles of CO_2 per liter, which is common terminology used in clinical chemistry. The conversion to concentration in terms of mg/100 ml brine, which has been used in our previous work (6,7), may be obtained as follows:

$$\text{mg } CO_2/100 \text{ ml brine} = \text{mmol } CO_2/\text{liter} \times 4.4$$

if the sample is analyzed directly. However, if the sample is stored in a Vacutainer containing 0.5 ml NaOH prior to analysis, as we recommend, the appropriate calculation is:

$$\text{mg } CO_2/100 \text{ ml brine} = \text{mmol } CO_2/\text{liter} \times 4.66$$

Note:

Concentrations of CO_2 in controlled fermentations (1) of cucumber brines may reach 80 to 100 mg/100 ml brine (ca. 18 to 23 mmol/liter) unless the brine is purged. Natural fermentations predominated by yeasts or high gas-forming bacteria may attain

values well over 100 mg/100 ml brine. It is recommended in the controlled fermentation process that the brine be purged with nitrogen gas to prevent the CO_2 concentration from reaching values over 20 mg/100 ml brine (1).

References Cited

1. Etchells, J. L., Bell, T. A., Fleming, H. P., Kelling, R. E., and Thompson, R. L. 1973. Suggested procedure for the controlled fermentation of commercially brined pickling cucumbers--the use of starter cultures and reduction of carbon dioxide accumulation. Pickle Pak Sci., volume 3, no. 1: 4-15.

2. Fleming, H. P., Thompson, R. L. and Etchells, J. L. 1974. Determination of carbon dioxide in cucumber brines. J. AOAC, volume 57: 130-133.

3. Harleco CO_2 Apparatus. Harleco, 60th Woodland Ave., Philadelphia, Pa. 19143.

4. Etchells, J. L. and Jones, I. D. 1946. Procedure for bacteriological examination of brined, salted and pickled vegetables and vegetable products. Am. J. Public Health, volume 36: 1112-1123.

5. Becton-Dickinson, Division of Becton, Dickinson and Company, Rutherford, New Jersey 07070. Tubes used herein were B-D No. 4710, 10 ml capacity, 16 x 100 mm.

6. Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E., and Bell, T. A. 1973. Bloater formation in brined cucumbers fermented by Lactobacillus plantarum. J. Food Sci., volume 38: 449-503.

7. Fleming, H. P., Thompson, R. L., Etchells, J. L., Kelling, R. E., and Bell, T. A. 1973. Carbon dioxide production in the fermentation of brined cucumbers. J. Food Sci., volume 38: 504-506.

Acknowledgments

R. G. Switzer, Western Canning Company, LaJunta, Colorado, has tested the Harleco CO_2 Apparatus independent of our work and confirmed that it is a practical, easy-to-use method for measuring CO_2 in cucumber brines.

Mention of a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture or the North Carolina Agricultural Experiment Station.

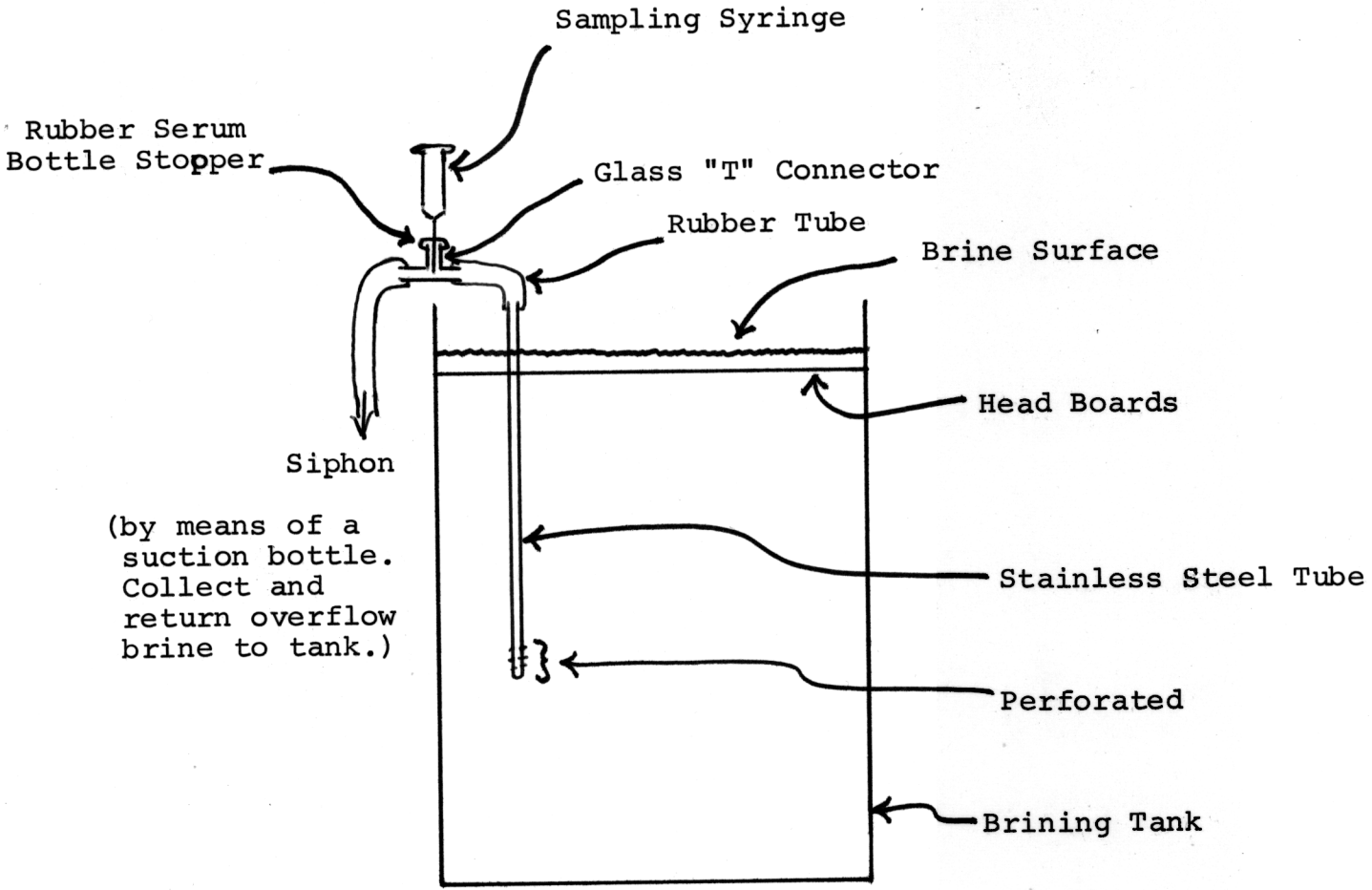
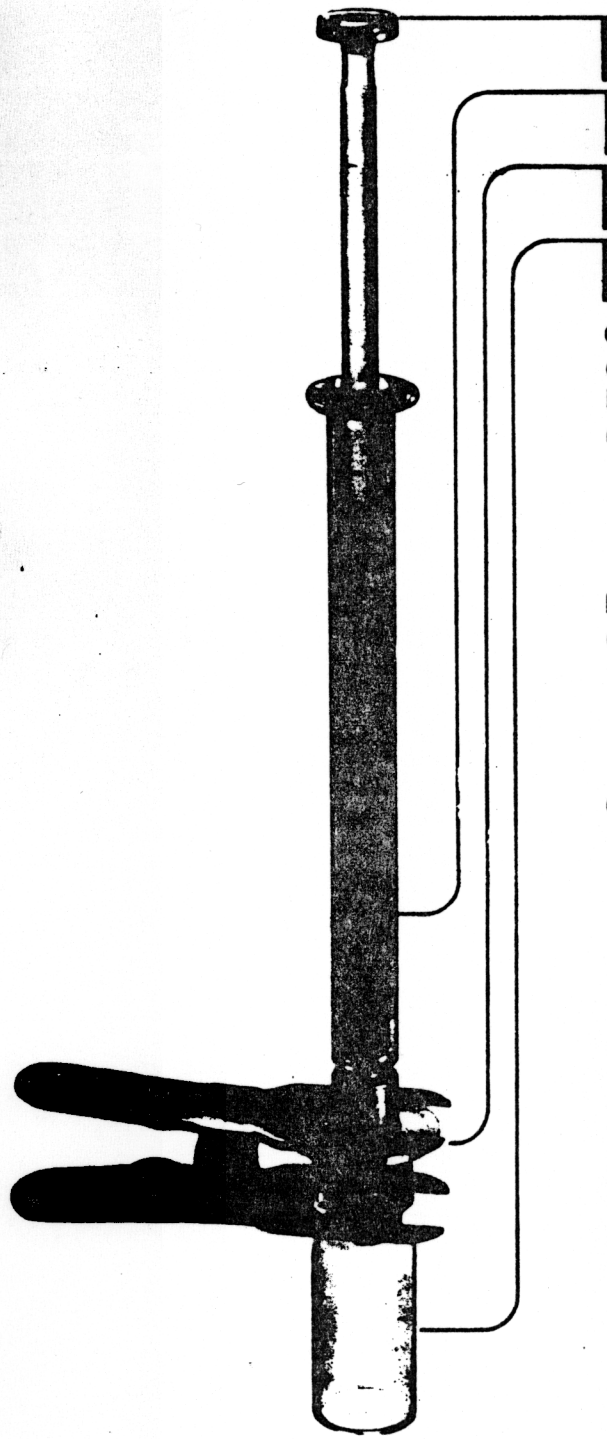


Figure 1. Device for withdrawing brine samples for CO_2 analysis from a cucumber brining tank. Not drawn to scale.

The Harleco CO_2 Apparatus Set measures CO_2 content (total CO_2 bicarbonate) in approximately one minute by means of a unique, direct determination. Only one pipetting is required.

Harleco® CO_2 apparatus†

Item 64887, price $48.50



CONTENTS:

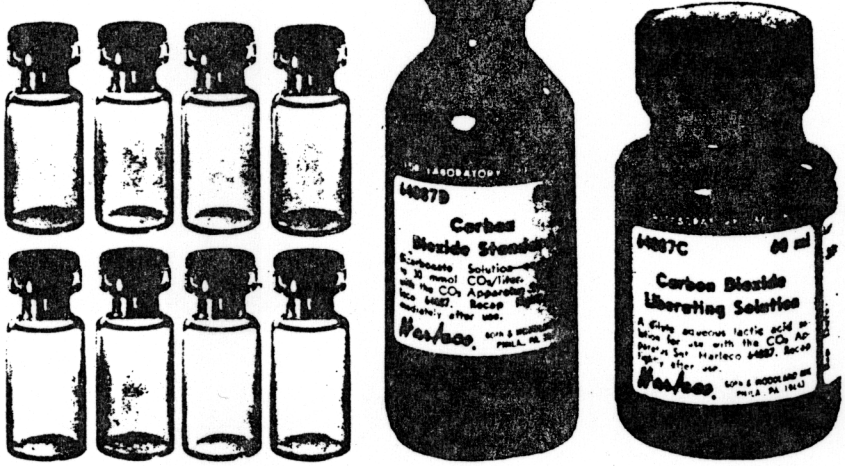
Carbon Dioxide Syringe, 1 each; Apparatus Clamp, 1 each; Reaction Vessels with Stoppers, 25 each; and the following:

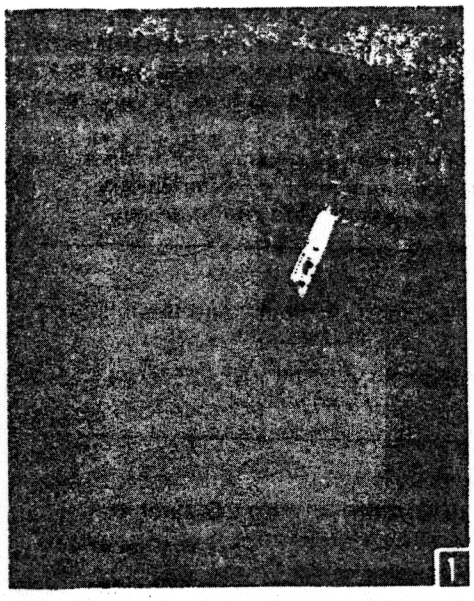
64887A CO_2 Reagent Set consisting of:
Carbon Dioxide Liberating Solution, dilute aqueous lactic acid 1 x 60 ml
Carbon Dioxide Standard, equivalent to 30 mmol CO_2/liter 1 x 60 ml

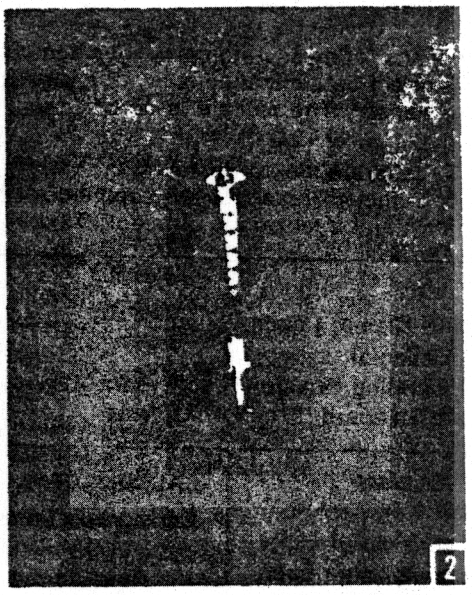
REPLACEMENT PARTS:

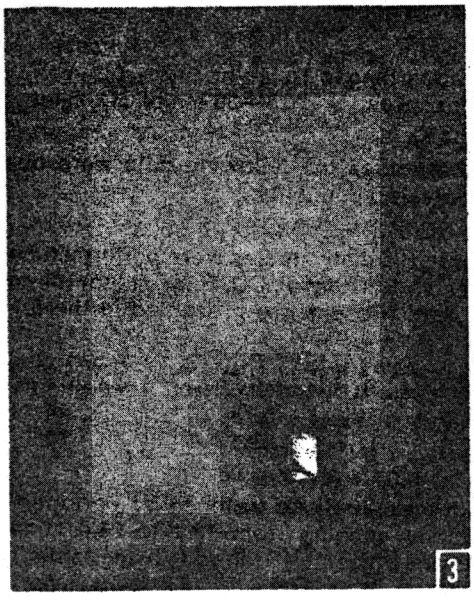
64887A CO_2 Reagent Set consisting of: $ 8.50
Carbon Dioxide Liberating Solution, dilute aqueous lactic acid 1 x 60 ml
Carbon Dioxide Standard, equivalent to 30 mmol CO_2/liter 1 x 60 ml

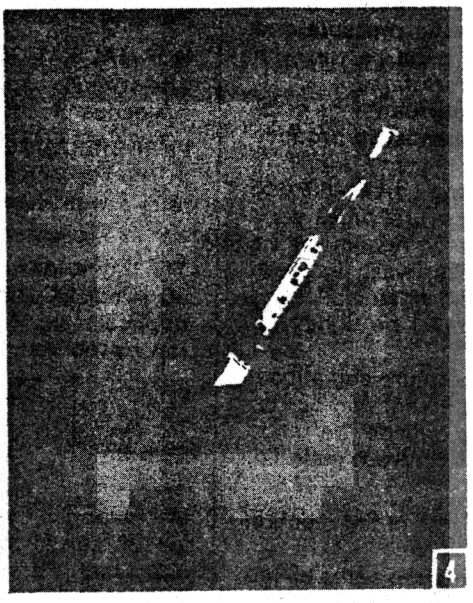
64887B CO_2 Reaction Vessel Set consisting of: $15.00
Reaction Vessels, 50 each
Reaction Vessels Stoppers, 50 each

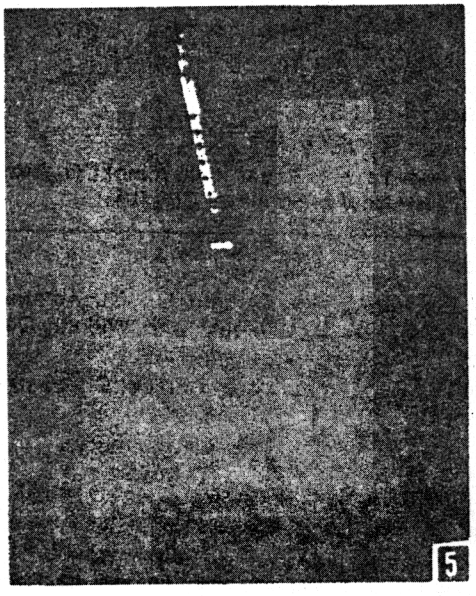


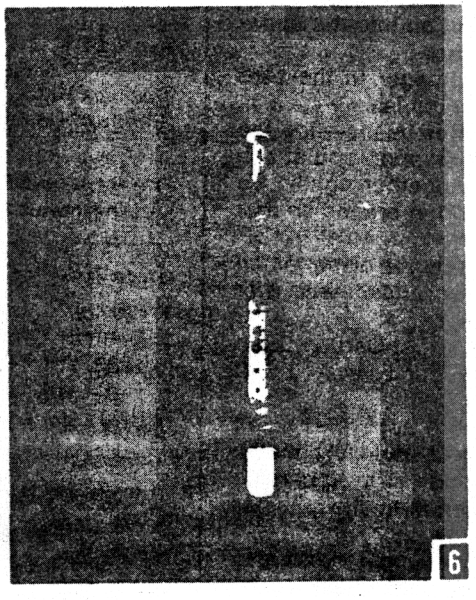












illustrated CO_2 apparatus procedure

Pipet 1.0 ml of serum into one of the sample vials supplied and stopper. **Treat the standard in similar fashion.**

1

Draw Carbon Dioxide Liberating Solution into the apparatus a few lines above the zero mark.

2

Invert the syringe and bring the plunger to the zero mark to allow for the escape of air bubbles.

3

Carefully assemble the CO_2 Apparatus by inserting the syringe tip into the reaction vessel stopper, and attach the clamp so that all units will fit together tightly. Be sure to keep the plunger of the syringe braced so that it cannot change position.

Caution: Care should be taken to slide the clamp on all the way to insure proper attachment.

4

Depress the plunger completely to expel Carbon Dioxide Liberating Solution into the reaction vessel. (Make sure plunger is free in syringe barrel.)

5

Mix the sample by shaking the entire apparatus at high speed on a Vortex mixer. Hold the clamp securely on either side of the syringe. The apparatus must be held upright in the mixer until the plunger stops rising (about 15 seconds).

Caution: Excessive pressure on the clamp handles may reduce clamping pressure and cause apparatus to come apart during mixing.

6

From the scale on the syringe record the reading at the bottom of the plunger to measure CO_2 displacement. Calculate CO_2 content using the equation shown on next page.

Harleco CO_2 apparatus

The Harleco CO_2 Apparatus provides a means for measuring carbon dioxide content (total CO_2, bicarbonate) in approximately one minute by means of a unique direct determination. Only one pipetting is required. The method used is the classical Van Slyke CO_2 content determination[1]. The sample is acidified with lactic acid and agitated to accelerate CO_2 release.

A sodium bicarbonate standard is used to calibrate the apparatus to the varying atmospheric pressure and temperature under which the test is being performed. Sufficient reagents are supplied for a minimum of 55 determinations if one standard per unknown is performed per day, and a maximum of about 275 if multiple samples are run at a time.

CONTENTS

Carbon Dioxide Syringe, 1 each; Apparatus Clamp, 1 each; Reaction Vessels with Stoppers, 25 each; and the following:

CO_2 Reagent Set consisting of:

Carbon Dioxide Liberating Solution, Lactic acid, 22% solution	1 x 60 ml
Sodium Bicarbonate Standard, 30 mmol CO_2/liter	1 x 60 ml

PRECAUTIONS

1. Store reagents at room temperature.
2. Use serum or plasma only.
3. Rinse and drain syringe with distilled water after each series of tests.
4. To compensate for varying atmospheric pressure and temperature, the 30 mmol standard **must** be run with each series of CO_2 determinations performed.
5. Good laboratory practice and clean surroundings combined with careful technique are essential to produce results of high quality.
6. Plunger must be lubricated by drawing carbon dioxide liberating solution into the syringe several times prior to use. If plunger does not move freely, results will be erroneous. If plunger becomes stuck, wash in warm Contrad 70 solution (Item 64695) and rinse well.
7. The sodium bicarbonate standard must be tightly recapped immediately after use.
8. If, under ordinary conditions of temperature and atmospheric pressure, the syringe displacement number for the 30 mmol CO_2/liter standard lies outside the range of 28-32 units, replace the reagents with fresh material.

PROCEDURE

1. Pipet 1.0 ml of serum or plasma into one of the reaction vessels supplied, and stopper.
   Important: Standard should be treated the same as the unknown.
2. Draw carbon dioxide liberating solution into the apparatus a few lines above the zero mark.
3. Invert the syringe and bring the plunger to the zero mark to allow for the escape of air bubbles.
4. Carefully assemble the CO_2 apparatus by inserting the syringe tip into the reaction vessel stopper, and attach the clamp so that all units will fit together tightly. Be sure to keep the plunger of the syringe braced so that it cannot change position.
   Caution: Care should be taken to slide the clamp on all the way to insure proper attachment with maximum clamping pressure.
5. Depress the plunger completely to expel Carbon Dioxide Liberating Solution into the reaction vessel (twist plunger to assure free movement).
6. Mix the sample by shaking the entire apparatus using a Vortex-Genie* mixer at full speed. Hold the clamp securely on either side of the syringe. The apparatus must be held upright in the mixer until the plunger stops rising (about 15 seconds).
   Caution: Excessive pressure on the clamp handles may reduce clamping pressure and cause apparatus to come apart during mixing.
7. From the scale on the syringe record the reading at the bottom of the plunger to measure CO_2 displacement. Calculate CO_2 content using the equation that follows.

*Registered Trademark of Scientific Industries, Inc.

CALCULATION

$$CO_2 \text{ content (mmol } CO_2\text{/liter)} = \text{mEq } CO_2\text{/liter} = \frac{\text{Reading of Unknown}}{\text{Reading of 30 mmol Standard}} \times 30 \text{ mmol } CO_2\text{/liter}$$

NORMAL RANGE

The normal range according to Tietz[2] is:

Venous plasma or serum	23—30 mmol CO_2/liter

PERFORMANCE DATA

In the Harleco laboratories the following data were obtained with manufactured reagents:

REPRODUCIBILITY

The day-to-day reproducibility studies were performed on 30 mmol CO_2/liter and 50 mmol CO_2/liter standards in duplicate for ten days. Results are presented in the following table:

Level	Average Value (mmol CO_2/liter)	Standard Deviation (mmol CO_2/liter)	Coefficient of Variation (%)
Normal	29.9	0.73	2.44
Elevated	47.6	0.81	1.70

COMPARISON

A comparison with the Van Slyke method was performed on 27 serum samples ranging from 12.1 to 26.4 mmol CO_2/liter. Results are presented in the following table:

	Van Slyke (mmol CO_2/liter)	Harleco CO_2 (mmol CO_2/liter)
Average	21.2	21.7

Bias ... 0.5

Standard Deviation ... ±1.87

LINEARITY

The Harleco CO_2 Apparatus is linear to 40 mmol CO_2/liter and the results are 5% low at 50 mmol CO_2/liter.

REFERENCES:

1. Peters, John P.; Van Slyke, Donald D.; Quantitative Clinical Chemistry, Vol. 2, Williams and Wilkins Co., 1932.
2. Tietz, Norbert W., Fundamentals of Clinical Chemistry, W. B. Saunders Co., 1970, p. 631.

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Diagnostic reagent — for professional use only.