
Longevity Testing of Calibration Gas Samples in QuinTron Evacuated Glass Tubes

Abstract

Gas samples can be collected and stored for long periods of time in evacuated glass tubes. QuinTron suggests samples are analyzed within one week after they are collected. The maximum acceptable holding time for a sample in a tube is two weeks. Otherwise, CO₂ absorption by the septum can cause inaccurate corrections of H₂ and CH₄ concentrations.

Introduction

When bacteria digest (or ferment) food substances, they produce acids, water and gases. The major gases produced by bacteria include, primarily, hydrogen (H₂), methane (CH₄), carbon dioxide (CO₂) and small concentrations of aromatic gases.¹ These gases are absorbed into the blood circulating near the site of digestion and are carried to lungs, where they are equilibrated with the air in the alveoli. When a patient exhales, gases contained in alveolar air can be captured and measured.

QuinTron manufactures instruments and accessories which are designed to capture and analyze the contents of a patient's breath sample. Specifically, our instrumentation measures concentrations of breath hydrogen (H₂) and methane (CH₄) in parts per million (ppm) and the percentage of exhaled carbon dioxide (CO₂). In the EasySampler breath collection system, a patient's breath sample can be captured in an evacuated glass tube. These kits are preferred by clinics and laboratories which analyze samples collected off-site and are then returned to the lab for analysis. Hydrogen, being the smallest element, can easily diffuse through many materials; one exception to this being glass. The stability of the sample, the ability to hold a sample for a long period of time, and the easy handling of the tube make it ideal for off-site collection and analysis.

EasySampler kits are meant to be analyzed with a BreathTracker model SC, H₂+, or CH₄+ or a Microlyzer model SC instrument. These instruments utilize a CO₂ correction factor that minimizes errors caused by improper sampling techniques. The main sources of error being the alveolar air sample is contaminated with either dead space (air contained in the trachea, the bronchi or bronchioles), or room air during collection. If the sample is contaminated it can be corrected based on the concept that CO₂ is present in alveolar air at a virtually constant concentration; while it is essentially absent in room air. Therefore, if alveolar air is erroneously mixed with room air, the concentration of CO₂ will be reduced; as will that of any trace gases present in the sample. By knowing the degree to which the CO₂ was diluted, it is possible to apply a correction to the analysis of the trace-gases as well, thus being able to calculate the true "alveolar" concentration of the sample which was contaminated.

QuinTron had previously used Labco Limited as an evacuated tube supplier. Before the BreathTracker instrument line was released, testing with these tubes showed unusually high H₂ readings due to excessive silicone contamination in the tubes. These problems do not affect the Microlyzer SC instrument, which has a 52" column which effectively traps any silicone affecting the H₂ and CH₄ readings.

Because of these tube compatibility issues with the BreathTracker instrument, QuinTron worked with a contract manufacturer to assemble our own evacuated glass tubes. These tubes are silicone free and evacuated as close to an absolute vacuum as possible. Sets of tubes are vacuum-packed in order to ensure the vacuum in the tubes stays intact. The purpose of this study was to determine how long these evacuated glass tubes can adequately hold a gas sample without significant losses in H₂, CH₄ and CO₂ concentration.

Materials/Method

Twenty evacuated glass tubes were filled with calibration gas. The calibration gas tank values were H₂ = 150ppm, CH₄ = 75ppm, CO₂ = 6.2%, with an uncertainty of ± 2 ppm for H₂ and CH₄ and $\pm 0.2\%$ for CO₂. The H₂, CH₄ and CO₂ concentrations in each tube were measured on a calibrated BreathTracker SC five at a time. Five tubes were analyzed the day they were filled, five one week later, five two weeks later and the last five three weeks after they were filled. All tubes were emptied via the SamplXtractor SX-2 instrument into the BreathTracker SC.

Results

Table 1 shows the tube number, the week each sample was measured, and the uncorrected and corrected H₂, CH₄ and CO₂ concentrations for that week. The left side of the table (Uncorrected Data) shows the H₂, CH₄ and CO₂ concentration before the CO₂ correction factor was applied. The right side of the table (Corrected Data) shows the H₂ and CH₄ concentrations corrected for dilutions along with the CO₂ correction factor.

Table 1: Uncorrected and Corrected Gas Concentrations of Each Sample Measured Week-by-Week

Tube #	Week	UNCORRECTED DATA			CORRECTED DATA		
		H2 (ppm)	CH4 (ppm)	CO2 (%)	H2 (ppm)	CH4 (ppm)	CO2 Corr Factor
1	0	153	76	6.3	151	75	0.99
2	0	151	76	6.3	149	75	0.99
3	0	151	76	6.4	149	75	0.99
4	0	151	76	6.4	146	74	0.97
5	0	149	75	6.4	145	73	0.97
6	1	149	75	5.9	159	79	1.05

7	1	148	75	5.9	155	79	1.05
8	1	147	74	5.8	156	78	1.06
9	1	149	75	5.9	156	79	1.05
10	1	147	75	6.0	151	77	1.03
11	2	142	73	5.4	162	83	1.14
12	2	141	73	5.5	158	82	1.12
13	2	142	73	5.5	159	82	1.12
14	2	141	73	5.6	155	80	1.10
15	2	140	72	5.5	157	81	1.12
16	3	138	73	5.3	160	85	1.16
17	3	136	73	5.3	158	85	1.16
18	3	140	73	5.5	157	82	1.12
19	3	141	73	5.5	158	82	1.12
20	3	140	72	5.4	160	82	1.14

Tables 2 & 3 show the percent errors for the Uncorrected and Corrected Data. After two weeks, there is over a 10% error for the CO₂ concentration. It is believed that the septum absorbs CO₂ over time. At two weeks, the average concentration of H₂ in the tubes was 141ppm uncorrected and 158ppm corrected. The average concentration of CH₄ was 73ppm and 82ppm when corrected for loss of CO₂. It is important to note that the majority of errors are related to changes in the CO₂ concentration. Percent errors at three weeks were not determined.

Table 2: Percent Errors of “Uncorrected Data” over Time

	Hydrogen	Methane	Carbon Dioxide
Week 0	0.67%	1.33%	2.58%
Week 1	1.33%	0%	4.84%
Week 2	6%	2.67%	11.3%

Table 3: Percent Errors of “Corrected Data” over Time

	Hydrogen	Methane	Carbon Dioxide
Week 0	1.33%	1.33%	2.58%
Week 1	3.33%	4%	4.84%
Week 2	5.33%	9.33%	11.3%

Based on the data above, QuinTron recommends a sample holding time of one week in the glass tubes.

Conclusion

QuinTron suggests samples are analyzed within one week after they are collected. The maximum acceptable holding time of a gas sample in the glass tubes is two weeks. Holding a sample longer than two weeks runs the risk of the septum absorbing too

much CO₂ which can lead to inaccurate H₂ and CH₄ corrections. If the sample has to be stored longer than two weeks, customers should consider not using the corrected values for H₂ and CH₄, using the uncorrected values (raw) instead. In addition, it is clear that H₂ is escaping (at a very low rate) from the tube over time. CH₄ is also escaping, but at a lower rate compared to H₂. Thus the combination and H₂ escape and CO₂ absorption increases the percent error of gas measurement after one week.

For further information on the history and science of breath-testing, sample protocols and collection techniques please reference Breath-Tests & Gastroenterology, 1998 edition, written by Lyle Hamilton Ph.D. or request information from QuinTron directly.

References

1. Bond, J.H., Levitt, M.D. Quantitative measurement of lactose absorption. *Gastroenterol.* 1976; 70(6):1058-62