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Note

Evacuated blood collecting tubes as containers for taking and storing gas samples

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Gas samples for analysis may be collected in containers which are variations of either tubing with a valve at each end or a syringe with gas-tight seals and a valve at the intake end. These are excellent for taking and delivering one sample at a time but are costly and inconvenient when taking large numbers of samples at remote locations. This report describes an evaluation of evacuated, rubber-stoppered glass tubes manufactured for blood collection as the vehicle for collection and storage of gas samples for gas chromatographic analysis.

Gas samples were analyzed by gas chromatography[1,2] using a Hewlett-Packard 7620 gas chromatograph fitted with thermal conductivity detector, liquid-CO_2 cooling and integrator. The chromatograph was fitted with dual 6 ft. $\times$ $\frac{1}{4}$ in. stainless-steel columns of a Porapak Q (80–400 mesh). The temperature program of the oven was: 4 min isothermal at −60°; temperature increase 30°/min to 90°, isothermal 2 min at 90°; temperature increase 30°/min to 170° and 4 min isothermal at 170°. Helium was used at the carrier gas. Gas samples (500 μl) were injected into the chromatograph using 500-μl gas-tight syringes fitted with closure valves between syringe and needle.

Gases used in this study were: He; CH_4 (natural gas, >99% pure CH_4) from laboratory gas cocks; 3.01% CO_2, balance N_2; and 11.91% CO_2, balance O_2. The latter two are normally used for calibration of blood gas analyzers*. Mixtures of these gases were prepared by downward displacement of water. Control gas samples at CO_2–N_2 or CO_2–O_2 mixtures and CH_4 were taken by direct sampling with the injection syringe from the wet electrode compartments of a Radiometer BEU-1 blood electrode unit.

Evacuated blood collecting tubes (Venoject®)** without anticoagulant were used. All tubes were from one manufacturing lot and were taken from laboratory stock. The tubes were filled with gas mixture using the standard tube holder fitted with a bleeding needle inserted into a contained gas mixture through a rubber injection stopper. The evacuated tube was pressed onto the adapter needle and gas was allowed to fill the tube to atmospheric pressure.

* Purchased from Gas Dynamics. Scarborough, Ontario, Canada.

** Manufactured by Jintan Terumo, Tokyo, Japan; distributed by Standard Hospital Supply, Calgary, Canada. Other makes include Vacutainor, Becton Dickinson, Mississauga, Ont., Canada; Monoject, Sherwood Industries, St. Louis, Mo., U.S.A.

TABLE I

REPRODUCIBILITY OF GAS ANALYSIS AND EFFECTS OF STORAGE

Sample and treatment	*No. of samples*	*Area of 500-μl peaks (μvolt/sec) ± S.D. (CV)*				*Gas composition (area corrected, %) ± S.D.*			
		N_2	O_2	CH_4	CO_2	N_2	O_2	CH_4	CO_2
Gas in blood tubes, He makeup	39	120000 ± 3340 (2.78)	36700 ± 2000 (5.39)	—	—	78.0 ± 2.2	22.0 ± 1.18	—	—
CH_4, free** (natural gas)	5	9400 ± 850 (9.06)	—	495000 ± 12500 (2.52)	—	1.2 ± 0.1	—	98.8 ± 2.5	—
From blood tubes	5	125200 ± 1040 (0.83)	37000 ± 1310 (3.54)	373000 ± 4270 (1.14)	—	17.0 ± 0.1	4.6 ± 0.2	78.4 ± 0.9	—
Blank corrected*		5200	300	373000	—	W0.9	—	99.1	—
3.01% CO_2–N_2, free**	10	630000 ± 10200 (1.62)	—	—	23000 ± 1336 (5.8)	97.0 ± 1.6	—	—	2.9 ± 0.2
From blood tubes	10	691700 ± 20700 (3.00)	36500 ± 1820 (4.98)	—	17560 ± 927 (1.72)	93.5 ± 2.8	4.5 ± 0.2	—	2.0 ± 0.1
Blank corrected*		571700	−200	—	17560	97.5	−0.1	—	2.6
11.91% CO–O, free**	10	—	619600 ± 9700 (1.57)	—	102800 ± 1765 (1.72)	—	87.1 ± 1.4	—	12.9 ± 0.2
From blood tubes	10	111000 ± 4600 (4.17)	516000 ± 10200 (1.98)	—	80250 ± 1530 (1.91)	17.0 ± 0.7	72.8 ± 1.4	—	10.1 ± 0.2
Blank corrected*		−9000	479300	—	80252	—	87.8	—	12.2
Mixed natural gas and 11.91% CO_2–O_2	14					21.1 ± 0.7 (3.41)	37.8 ± 0.8 (2.25)	38.2 ± 0.4 (1.07)	3.9 ± 0.1 (3.9)
Stored at 20° for 7 days	8					20.6 ± 0.8 (4.1)	37.3 ± 0.5 (1.42)	38.5 ± 0.5 (1.36)	3.8 ± 0.1 (1.84)
Stored at 20° for 14 days	10					42.6 ± 18.4 (43.2)***	31.4 ± 5.4 (17.2)***	23.7 ± 12.3 (51.9)***	2.4 ± 1.1 (46.1)
Stored at −20° for 14 days	7					20.7 ± 0.5 (2.61)	37.2 ± 0.6 (1.69)	38.9 ± 0.4 (1.13)	3.6 ± 0.1 (3.41)

* Blank values for area comparisons was gas in blood tube; for % composition, N_2 and O_2 values in CH_4 tubes were used as blanks.

** Collected from the BEU-1 flow cells.

*** Significant difference from original $P < 0.02$.

Test gas samples were drawn into blood collecting tubes from a constantly flowing gas stream flowing through a pair of bubble jars. Samples were drawn from the first bubble jar. The second jar provided a seal to prevent aspiration of air into the system during the sudden filling of the evacuated tubes.

Calibration curves for N_2, O_2, CO_2, and CH_4 were prepared by analyzing 500-, 400-, 300-, 200-, 100- and 50-μl samples of air, 11.91% CO_2–O_2 and CH_4. The response of the instrument was linear between 4 and 12% CO_2 and linear for N_2, O_2 alone, O_2 in presence of N_2*, and CH_4 between 1 and 70–100% composition. The relative responses of the detector relative to N_2 was calculated from the slopes to be: 1.1 for O_2 alone; 0.92 for O_2 in the presence of N_2*; 1.55 for CH_4; and 0.82 for CO_2.

The reproducibility of the gas analysis itself was tested by analyzing 44 samples of laboratory air taken over a 7-day period. The area values for N_2 and O_2 were for N_2 and O_2 were 596200 $\pm$ 11700 and 172000 $\pm$ 2450, respectively, with coefficients of variation (CV) of 1.95 and 1.43. These correspond to air composition values of 76.1 $\pm$ 1.5% N_2 and 20.9 $\pm$ 0.3% O_2.

The reproducibility of blank values (*i.e.*, the residual air in the evacuated blood tubes) was tested by filling tubes with wet He and analyzing the contained gases**. The CV of N_2 and O_2 for the 39 tubes were 2.78 and 5.39, respectively (Table I).

Analysis of the CH_4 sampled from the BEU-1 showed the presence of small proportions of N_2 (1.91% of CH_4 integrated peak area) which had a CV of 9.06% and CH_4 with a CV of 2.52%. When CH_4 was sampled using blood tubes, the N_2, O_2 and CH_4 measured (Table I) had CV values of 0.83, 0.35 and 1.14%, respectively. The known gas mixtures were analyzed as controls on 10 different days during the course of the trials described. The CV for the analysis of 3.01%-N_2 mixture were 1.62 and 5.81 for N_2 and CO_2, respectively and for the 11.91% CO_2–O_2 mixture were 1.57 and 1.72% for O_2 and CO_2, respectively.

Storage of a mixture of CH_4 and 11.91% CO_2–O_2 (Table I) resulted in no significant changes in the composition after 7 days at 20° but significant ($P < 0.01$) changes were observed after 14 days and the reproducibility of values between tubes was very poor. The change appeared to be an exchange of CH_4 for air. At —20° there were no significant changes after 14 days.

Through these trials a water peak of variable size appeared in all chromatograms. Attempts to quantitate the water proved futile as no reproducibility was obtained. However, as the analysis was being developed for use with rumen samples[3] and as reproducible results were obtained with water-saturated gas samples, the water content was considered a constant and was ignored. Using a different analytical system, it may be possible to estimate the water content.

The shelf life of the blood tubes will have an effect upon analytical results. Experience in drawing blood with these tubes indicates that the vacuum is reduced or lost with time. For this reason, only fresh tubes should be used for gas collection, tubes for each sample group should have the same lot number and blank values should be estimated with each analysis.

The data presented demonstrate the feasibility of using evacuated blood

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* Chromatograms of N_2 + O_2 gave overlapping peaks. The O_2 peak follows the N_2.

** One lot of tubes assayed in 1972 contained 0.5–1.0% CO.

collecting tubes for collecting, transporting, and briefly storing gas samples for analysis. Reproducibility of analysis was good and the amounts of gas originally present in the tubes could be reliably subtracted as an analytic blank. For gases of 5% (v/v), a CV of 1–2% was easily attained. Gases in smaller proportion (1–5%) were estimated with CV of less than 6%. Where this variability is acceptable, the blood tubes provide an inexpensive, convenient method for handling gas samples, particularly when large numbers of samples are to be collected at remote sites.

CONCLUSION

Evacuated blood collecting tubes were tested for collection, transport and short term storage of gas samples for gas chromatography. Using a carrier-gas filled tube as a blank analysis of major components of air and rumen head-space gas could be achieved with CV $<5\%$.

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REFERENCES

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