

Air bubbles and temperature effect on blood gas analysis

G MADIEDO, R SCIACCA, AND L HAUSE

From the Department of Pathology, the Medical College of Wisconsin, Milwaukee, Wis, USA

SUMMARY The effect of temperature, time of storage, and presence of air bubbles in specimens for blood gas analysis was studied. The results show that air bubbles in a 10% proportion are undesirable because of significant elevation in the PO_2 , and the storage of anaerobic blood samples at room temperature (25°C) is acceptable when measurements are done within the first 20 minutes.

The presence of air bubbles in blood samples for blood gas analysis can cause significant error in such determinations.^{1,2} Although the potential of such error has been recognised, the clinical significance is not clear since most of the available data have been obtained from theoretical and experimental models.¹⁻⁴ In this study we have analysed the observable effects of air bubbles introduced into blood samples and the time-temperature effects of storage. The results of the study show some of the actual effects of air bubbles and storage on routine blood gas determination.

Material and methods

Blood samples were obtained from patients in the intensive care unit and coronary care unit of Milwaukee County Hospital by arterial puncture in a 6 ml disposable plastic syringe (Monoject^(R)) containing a lead washer and approximately 0.2 ml of sodium heparin (1000 IU/ml). The volume of blood obtained was approximately 4-5 ml. Any entrapped air bubbles were removed immediately and the initial blood gas analysis was performed within 45 seconds using a Radiometer ABL-1 blood gas analyser. The syringes were sealed with a rubber cup and divided into three groups. In the first group an air bubble corresponding to 10% of the blood volume was introduced into the syringe, which was manually inverted 10 times and placed in ice (4°C) for 15-20 minutes; then blood gases were measured. The second and third groups were kept anaerobically, one at 4°C and the other at room temperature (25°C). Blood gases were determined at different intervals up to 120 minutes after the initial measurement. Comparative analysis of these results

was then performed based on the difference observed between the initial result and subsequent results from each sample. The statistical significance of differences was determined with a paired *t* test and the comparison of differences in the precision of the respective method was used for clinical appraisal of the various treatments.

Results

Those samples exposed to an air bubble equivalent to 10% of the blood volume and kept at 4°C for 20 minutes showed an increase in PO_2 ranging from 1.7 to 29 mmHg with a mean increase of 11 mmHg. The increase was statistically significant ($p < 0.001$) (Fig. 1). The changes were greater in the specimens with the higher initial PO_2 values. In contrast, the specimens maintained anaerobically for 20 minutes at 4°C were considerably more stable, showing a

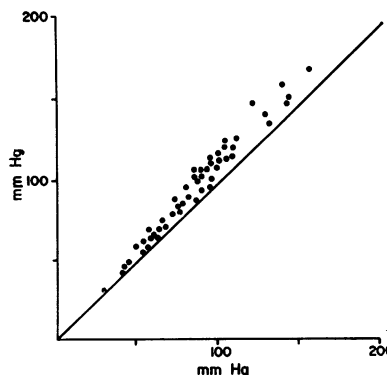


Fig. 1 Changes in PO_2 after blood exposure to air bubbles for 20 minutes at 4°C. Abscissa, initial values; ordinate, final values.

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mean PO_2 increase of only +1.7 and were not statistically different from the initial values ($P > 0.3$) (Fig. 2). The values for pH and PCO_2 were remarkably stable with or without the presence of air bubbles (Table) during the same 20-minute period.

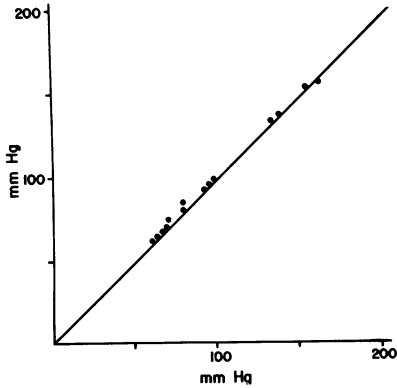


Fig. 2 Changes in PO_2 after blood storage in anaerobic conditions for 20 minutes. Abscissa, initial values; coordinate, final values.

Effect of anaerobic blood storage and equilibration of blood with air bubbles at 4°C for 20 minutes: mean difference (12 to 15 samples) \pm 2 SD

	Samples equilibrated with air bubble at 4°C	Samples kept anaerobically at 4°C
pH	- 0.0009 \pm 0.014	- 0.0015 \pm 0.006
PCO_2	- 0.18 \pm 1.54	+ 0.019 \pm 0.80

Figures 3-5 represent the differences in PO_2 , PCO_2 , and pH between the initial results (45 seconds) in samples kept anaerobically at 4°C and 25°C for 120 minutes. Each point represents the average difference of 12 to 15 samples at respective times with a \pm 2 SD range. The PO_2 increased when the samples were kept at 4°C, showing a mean difference of +2.98 \pm 2.91 at 30 minutes, but decreased at 25°C with a mean difference of -1.85 \pm 3.28 at 30 minutes. The variability (SD) among samples was higher at room temperature and increased progressively with time at both 4°C and 25°C. However, the variability increase was greater for the room temperature samples. The initial PO_2 values were within a range of 35-161 mmHg (Fig. 3).

The initial PCO_2 levels ranged between 23 and 77 mmHg. Pressures remained very stable when stored anaerobically at 4°C but suffered a slight increase at 25°C, accounting for a mean difference of +1.015 \pm 0.92 at 30 minutes. The variability among samples stored at room temperature was larger than in the samples at 4°C (Fig. 4).

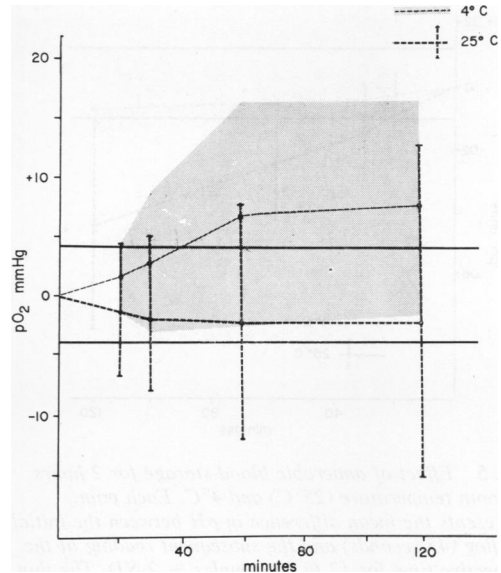


Fig. 3 Effect of anaerobic blood storage for 2 hours at room temperature (25°C) and 4°C. Each point represents the mean difference in PO_2 between the initial reading (45 seconds) and the subsequent reading at the respective time for 12 to 15 samples \pm 2 SD. The thin broken line and shaded area represent the measurements at 4°C, and the thick broken lines at 25°C. The horizontal solid lines indicate the method precision.

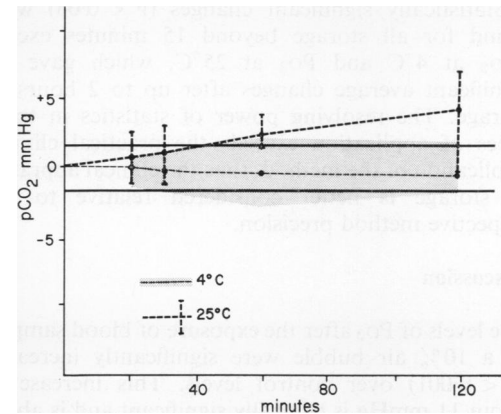


Fig. 4 Effect of anaerobic blood storage for 2 hours at room temperature (25°C) and 4°C. Each point represents the mean difference in PCO_2 between the initial reading (45 seconds) and the subsequent reading at the respective time for 12 to 15 samples \pm 2 SD. The thin broken line and shaded area represent the measurement at 4°C, and the thick broken lines at 25°C. The horizontal solid lines indicate the method precision.

The pH (Fig. 5) values decreased with both treatments, with a mean difference at 30 minutes of -0.0031 \pm 0.0042 and -0.014 \pm 0.0081 at 4°C and

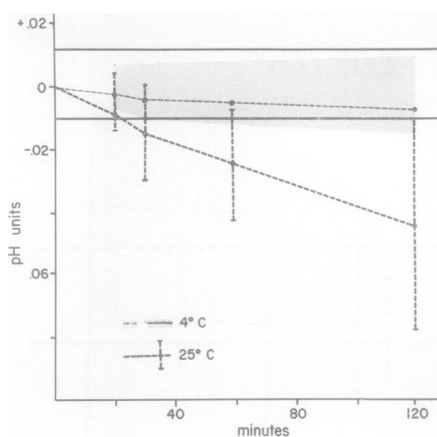


Fig. 5 Effect of anaerobic blood storage for 2 hours at room temperature (25°C) and 4°C. Each point represents the mean difference in pH between the initial reading (45 seconds) and the subsequent reading at the respective time for 12 to 15 samples \pm 2 SD. The thin broken line and shaded area represent the measurements at 4°C, and the thick broken lines at 25°C. The horizontal solid lines indicate the method precision.

room temperature, respectively. Variability among samples was considerably higher in the samples kept at 25°C. Initial pH levels were between 7.308 and 7.557.

Statistically significant changes ($P < 0.05$) were found for all storage beyond 15 minutes except PCO_2 at 4°C and PO_2 at 25°C, which gave no significant average changes after up to 2 hours of storage. The resolving power of statistics in these types of application exceeds the practical clinical application of the method; thus the clinical appraisal of storage is better considered relative to the respective method precision.

Discussion

The levels of PO_2 after the exposure of blood samples to a 10% air bubble were significantly increased ($P < 0.001$) over control levels. This increase of about 11 mmHg is clinically significant and is about 2.5 times the precision (2 SD) for the PO_2 method. These results are in agreement with the findings of Ishikawa *et al.*² after gently shaking the specimen at 4°C, in contrast to their findings at room temperature after violent agitation. Our results demonstrate progressively increased changes in PO_2 for respectively higher PO_2 levels. This phenomenon agrees with the findings of others² and probably results from the difference in haemoglobin saturation.⁴ Several of these specimens demonstrated oxygen pressures above atmospheric levels after

reaching equilibrium, a phenomenon which was predicted by Mueller *et al.*¹ in their experimental model. The increased solubility of O_2 in plasma at lower temperature and the fact that the PO_2 measurement is done at 37°C in a closed system explain the above observation.⁴ It is of interest that the observed changes of PO_2 occurred mainly within the first minutes. Samples that were followed for up to 1.5 hours (data not shown) gave minimal additional changes after the initial 20 minutes of storage.

The anaerobic storage of the blood at 4°C revealed high stability for pH and PCO_2 . The average differences of pH and PCO_2 during storage were low and within the respective precision levels established for each analysis. On the other hand, anaerobic storage at room temperature caused an increase in PCO_2 and a decrease in pH, which exceeded the method precision and which probably resulted from leucocyte metabolism, as postulated by Sigaard Anderson.⁵ Although the bias and variability among samples stored at room temperature progressively increased with the time of storage, there is no clinical significance to changes observed for the first 20 minutes.

The changes in PO_2 levels during anaerobic storage in the first 30 minutes are within the range of the method precision. After this time the variability among samples increases to unacceptable levels at both 4°C and room temperature. Factors such as cellular metabolic activity are probably responsible for the changes at room temperature while syringe wall permeability or the presence of undetected small air bubbles could be the cause for the changes observed at 4°C.

These results show that the presence of air bubbles in blood samples can cause significant elevation in PO_2 levels which are clinically important. Anaerobic storage at 4°C and at room temperature produces drift and/or variability in PO_2 levels which are outside the range of the method precision and consequently clinically significant after 30 minutes of storage. Changes in pH and PCO_2 in samples stored anaerobically at 4°C for up to 2 hours are smaller than the method precision. On the other hand, when stored at 25°C the changes exceed the method precision after 20 to 30 minutes. Variability in pH, PCO_2 , and PO_2 among samples increases with the time of storage and is larger at room temperature. Finally, the absence of clinically significant changes in pH, PCO_2 , or PO_2 (changes lower than method precision limits) during the first 20 minutes of anaerobic storage at either 4°C or 25°C support the clinical acceptability of storage of blood gas samples at room temperature.

We conclude that it is of great importance that the collection and handling of blood samples is done

in anaerobic conditions to obtain reliable PO_2 values, and, secondly, that the measurement of pH, PCO_2 , and PO_2 must be done within 20 minutes of sample collection in order to ensure reliable results.

References

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Requests for reprints to: Dr G Madiedo, The Medical College of Wisconsin, Milwaukee County Medical Complex, 8700 West Wisconsin Avenue, Milwaukee, Wisconsin 53226, USA.