A Stable Blood Product for pH–Blood-Gas Quality Control

Michael C. Steiner, Barry A. Shapiro, John Kavanaugh, John R. Walton, and Wayne Johnson

We describe how to prepare, store, and use a hemolyzed blood product for simultaneous pH, \( p_{CO_2} \), and \( PO_2 \) quality control. Tonometry of the blood product with two oxygen and two carbon dioxide concentrations resulted in consistent and reproducible values during 38 weeks. The resulting pH values were consistent and reproducible, demonstrating the metabolic acid–base stability of the blood product. We conclude that the proper preparation, storage, and use of the product results in consistent, reproducible, and economical quality control for pH, \( p_{CO_2} \), and \( PO_2 \) blood measurements.

Additional Keyphrases: blood gases • acid–base balance • \( p_{CO_2}, PO_2, pH \) • tonometry

The clinical need for accurate and economical quality control for pH, \( p_{CO_2} \), and \( PO_2 \) measurements has long been recognized (1, 2). Whole-blood samples with predictable values for pH, \( p_{CO_2} \), and \( PO_2 \) would be the ideal quality control but whole-blood tonometry has proven reliable only for \( p_{CO_2} \) and \( PO_2 \) (1, 2).

Serum (3) and lyophilized serum preparations have been developed for pH and \( p_{CO_2} \) controls but appear to be unreliable for \( PO_2 \). Although methods with aqueous solutions have been developed to provide controls for all three measurements (4–6), the probability that whole-blood pH controls significantly differ from aqueous pH controls places the usefulness of the aqueous preparations in some doubt (2, 7). There is a need in clinical blood-gas measurements for a stable reference material that more closely simulates blood. This report describes a hemolyzed blood preparation that provides reliable, economical and accurate controls for pH, \( p_{CO_2} \), and \( PO_2 \) measurements in blood.

Methods

Preparation and Storage of the Blood Product

Outdated packed erythrocytes were stored over a 6-month period at \(-20^\circ C\) until 59 pints were accumulated. These frozen units were simultaneously allowed to warm to room temperature during a 5-h period and then run through a blood filter into a 37.5-liter plastic jug. To restore the hemoglobin concentration to more normal values, 21 liters of sterile isotonic saline (25 \( ^\circ C \)) was added to the approximately 15 liters of thawed packed erythrocytes. The resulting hemolysate contained a hemoglobin concentration of approximately 80 g/liter with an erythrocyte count of approximately 90,000/mm\(^3\). Thus, at least 90% of the cells were hemolyzed in the preparation of the blood product.

One hundred thousand USP units of sodium heparin\(^2\) and 2880 mg of gentamicin sulfate\(^3\) were added to the hemolysate. To achieve a near-normal metabolic acid–base state, sufficient sodium bicarbonate powder was added until a base excess of approximately \(-4\) milliequivalents per liter was obtained. The resulting preparation was then aspirated into individual evacuated blood-collection tubes\(^4\) in 7-ml aliquots. The tubes were then placed in a freezer, where their contents required about 48 h to freeze completely.

Quality Control Protocol

We equilibrated the previously prepared and stored hemolyzed blood product with gas by means of a thin-film tonometer (IL 237)\(^5\) for 20 min at gas flow rates between 300 and 400 ml/min (8). A vial of frozen blood product was immersed in tepid water (approximately \(35^\circ C\)) for 10–15 min. The thawed blood product was then equilibrated with one of two gravimetrically produced gas mixtures (Table 1). The blood product was then aspirated into the electrode chambers of two or more pH/blood-gas analyzers via a 35-cm microbore polyvinyl chloride tubing. To minimize sampling contamination, the electrodes were exposed to an initial aliquot for 3–5 s, followed by a second aliquot for measurement.

We did this procedure at least three times during an 8-h work shift. A fresh vial of blood product was used

\(^1\) Versatol Acid Base; General Diagnostics, Division of Warner Lambert Co., Morris Plains, N.J. 07950.

\(^2\) Heparin Sodium Injection; The Upjohn Co., Kalamazoo, Mich.

\(^3\) "Garamycin" (Injectable); Schering Pharmaceutical Corp., Puerto Rico.

\(^4\) Vacutainer Tubes (3204 QS) containing 0.07 ml of a 150 g/liter solution of ethylenediaminetetraacetate; Becton-Dickinson and Co., Rutherford, N.J. 07070.

for each procedure. Gases T₁ and T₂ were alternated, T₁ always being the first gas used in each 8-h shift.

Calibration of pH and Blood Gas Electrodes

Four IL Model 513 pH/blood-gas analyzers with standard electrodes were calibrated to the appropriate slope values at least once every 8 h. The pH electrodes were calibrated with the manufacturer's standard buffer solutions (Instrumentation Laboratories, Inc.); pCO₂ and pO₂ electrodes were calibrated by using two gas mixtures. The temperature of each electrode and of the tonometer was maintained at 37 ± 0.2 °C, as measured by a Yellow Springs Instrument telethermometer.

Statistical Calculations

Each work shift, we calculated predicted gas tensions at body temperature and pressure for gases T₁ and T₂, using direct barometric readings, because both barometric pressure and gravimetrically produced gas-cylinder concentration varied over the 38-week period. Mean values and their standard deviations were calculated and are expressed as predicted gas tensions. Means, standard deviations, and regression analysis were computed by a commercially available calculator method (Olivetti Corp.).

To minimize errors attributable to technique and instrumentation, we defined limits of acceptability for data inclusion (Table 2). Criteria for these limits were arbitrarily set as a result of our empirical observation that larger errors were invariably attributable to techniques associated with the tonometry process (such as new personnel) or faulty electrodes.

Results

A minimum of three sets of measurements was accomplished for 798 consecutive 8-h work shifts, representing 22 113 measurements during a 38-week period.

Fig. 1. Mean pH measurements during 38 weeks. White dots represent the calculated mean for that week's pH measurements. One standard deviation from the mean is represented by black bars.

Fig. 2. Mean pCO₂ measurements during 38 weeks. White dots represent the calculated mean for that week's pCO₂ measurements. One standard deviation from the mean is represented by black bars. The heavy horizontal line at pCO₂ 70.0 mmHg for gas T₁ and pCO₂ 39.0 mmHg for gas T₂ represents the calculated mean for the 38-week test period.

Fig. 3. Mean pO₂ measurements during 38 weeks. White dots represent the calculated mean for that week's pO₂ measurements. One standard deviation from the mean is represented by black bars. The heavy horizontal line at pO₂ 70.0 mmHg for gas T₁ and pO₂ 39.0 mmHg for gas T₂ represents the calculated mean for the 38-week test period.

Statistical analyses of these measurements are summarized in Table 3.

Figures 1, 2, and 3 show weekly means for pH, pCO₂ and pO₂ measurements for gases T₁ and T₂. The heavy lines in Figures 2 and 3 represent the predicted mean pCO₂ and pO₂.

Discussion

A quality-control medium must be accurate (within the limits of the instrumentation), consistent, and reproducible. To assess the accuracy of our blood product, we included in the experimental design the predicted mean values for pCO₂ and pO₂ accounting for variability due to barometric pressure and standardized gases. In addition, wide limits of data acceptability were adopted

Table 1. Gas Mixtures for Tonometry

<table>
<thead>
<tr>
<th>Gas</th>
<th>CO₂ volumes per 100 volumes</th>
<th>O₂</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>10.0</td>
<td>5.6</td>
<td>84.4</td>
</tr>
<tr>
<td>T₂</td>
<td>5.6</td>
<td>20.0</td>
<td>74.4</td>
</tr>
</tbody>
</table>

Table 2. Limits of Data Acceptability

<table>
<thead>
<tr>
<th>Gas</th>
<th>pH</th>
<th>pCO₂</th>
<th>pO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>7.19 ± 0.05</td>
<td>68 mmHg ± 5 mmHg</td>
<td>40 mmHg ± 5 mmHg</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.05</td>
<td>38 mmHg ± 5 mmHg</td>
<td>138 mmHg ± 10 mmHg</td>
</tr>
</tbody>
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*1 mmHg = 133 Pa.

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Gas mixtures used for calibration: (C₁, 5.6% CO₂, 20.0% O₂, 74.4% N₂) and (C₂, 10.0% CO₂, 0.0% O₂, 90.0% N₂), gravimetrically produced by Linde Specialty Gas Plant, Division of Union Carbide Corp., East Chicago, Ind. 46312.
so that excluded data were unquestionably due to technical or human error. Since the differences between predicted and observed values (Table 3) are small (<3%), they can be attributed to instrumentation variability, instrumental design, and $P_O_2$ factors (9). The blood product's consistency for $P_CO_2$ and $P_O_2$ is demonstrated by the small standard deviations during 38 weeks (Table 3); the reproducibility is illustrated in Figures 2 and 3. Thus, we conclude that our blood product is accurate, consistent, and reproducible for $P_CO_2$ and $P_O_2$ when properly tonometered.

For the blood product to be a pH quality control, its pH–$P_CO_2$ relationship must remain stable throughout the storage period, i.e., metabolic activity during storage would alter this relationship. At consistent $P_CO_2$ values the observed pH values (Figure 1) were reproducible throughout the 38 weeks. As a further assessment of the long-term metabolic stability of the blood product, a linear regression analysis (weekly mean base excess vs. time) reveals that 200 weeks of storage would alter the base excess by no more than 1 milliequivalent per liter.

We conclude that proper preparation, storage, and use of this blood product results in an accurate, reproducible, stable, and simultaneous quality control for pH, $P_CO_2$, and $P_O_2$ measurements in blood.

References