

# Effects of Syringe Material, Sample Storage Time, and Temperature on Blood Gases and Oxygen Saturation in Arterialized Human Blood Samples

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**BACKGROUND:** The practice of on-ice storage of arterial-blood samples in plastic syringes for delayed analysis continues, and the effects of storage time and temperature on the measurement of blood-oxygen-saturation values ( $S_{aO_2}$ ) have not been adequately described. **OBJECTIVE:** To determine the effects of syringe material, storage time, and storage temperature on normal arterialized blood gas and  $S_{aO_2}$  values. **METHODS:** We used a temperature-controlled extracorporeal circuit to "arterialize" 500 mL of fresh, whole human blood at 37°C, and we used certified calibration gases of 12%  $O_2$  and 5%  $CO_2$  to produce normal blood-gas values. From that arterialized blood we took 90 samples and randomly assigned them to 6 groups, until there were 15 samples in each group. The groups were (1) plastic syringe, analyzed immediately, (2) plastic syringe, stored 30 min at 0–4°C, (3) plastic syringe, stored 30 min at 22°C, (4) glass syringe, analyzed immediately, (5) glass syringe, stored 30 min at 0–4°C, and (6) glass syringe, stored 30 min at 22°C. **RESULTS:** Compared to the samples that were analyzed immediately, the  $P_{O_2}$  of the samples stored in plastic syringes for 30 min at 22°C and at 0–4°C was significantly higher, with a clinically important magnitude of 11.9–13.7 mm Hg. The  $P_{CO_2}$  of blood stored in glass for 30 min at 0–4°C was significantly lower, although the magnitude of the difference (1.5 mm Hg) was not clinically important. There were no statistically significant differences in pH or oxygen saturation among the 6 groups. **CONCLUSION:** For accurate arterial-blood-gas results, samples drawn in plastic syringes should be analyzed immediately. If the analysis is going to be delayed, the samples should be drawn and stored in glass. *Key words:* blood gas analysis, syringes, temperature, time factors, specimen handling, glass, plastics, blood specimen collection. [Respir Care 2006;51(7):732–736. © 2006 Daedalus Enterprises]

## Introduction

The analysis of arterial blood gases is essential in assessing the oxygenation and ventilatory status of many

patients with cardiopulmonary problems. There are no more definitive measurements than arterial-blood-gas values when assessing the need for respiratory therapy. Pre-analytical errors in arterial-blood-gas analysis can adversely affect patient-care decisions when the magnitude of the error is clinically important. The American Association for Respiratory Care has adopted clinical practice guidelines that advocate best practices for sampling, handling, and analyzing arterial blood samples.<sup>1,2</sup> There is consensus that

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samples should be analyzed as soon as possible, and no longer than 30 min after sampling; however, there is con-

flicting evidence regarding on-ice storage of samples and the impact of storing samples in plastic. The Clinical and Laboratory Standards Institute recommends that samples taken in plastic syringes should not be iced, but instead should be kept at room temperature and analyzed within 30 min after collection, and that glass syringes should be used when analysis will be delayed for longer than 30 min.<sup>3</sup> Some respiratory care textbooks continue to advocate icing without restriction.<sup>4,5</sup> There is also a lack of consideration in the respiratory literature on the effects of syringe material, sample-storage time, and temperature on oxygen-saturation measurements.

Since the creation of plastic syringes in the early 1970s, there have been multiple studies of the effects of storage time and temperature on blood-gas measurements.<sup>6–11</sup> The conclusions regarding measurements of pH,  $P_{CO_2}$ , and  $P_{O_2}$  have not been consistent, and the different conclusions may be due to different study designs, different blood-sample volumes, different initial oxygen levels, and lack of control of syringe designs, syringe sizes, and hemoglobin concentrations. Some investigators used hospitalized patients and performed arterial punctures, whereas others obtained blood from indwelling catheters. Some used tonometers to create arterialized blood. Some did not analyze their initial samples immediately, because there was a delay while the samples were transported to the laboratory, which may have allowed unforeseen changes to the samples. Several researchers performed repeat analysis on leftover blood samples, which may have introduced error. How the syringes were iced was often not described. No study we found reported the measurement of oxygen saturation. In recent years, blood-sample volumes have decreased, the technology of blood-gas analysis has become automated, and point-of-care analysis and hemoximetry have become more widely available. We studied the effects of syringe material, 30 min of sample storage time, and 2 different storage temperatures on normal values of arterial blood gases and oxygen saturation, in a laboratory controlled environment, using commercially available arterial-blood-sampling devices.

### Methods

This study was performed at Ohio State University, Columbus, Ohio.

We obtained 500 mL of fresh, whole human blood, prepared with citrate phosphate dextrose anticoagulant, from the American Red Cross. We sampled blood, measured pH, and adjusted the pH to the normal range with sodium bicarbonate. We assembled an extracorporeal circuit to arterialize the blood. We produced arterialized human blood, using a temperature-controller set at 37°C, certified calibration gases of 12%  $O_2$  and 5%  $CO_2$  at 1.5–2.0 L/min through an oxygenator, and a centrifugal blood

pump-speed controller (Bio-Console 540, Medtronic Bio-Medicus, Eden Prairie, Minnesota) set at 600 mL/min, to create blood with an expected  $P_{O_2}$  of 91 mm Hg and  $P_{CO_2}$  of 37 mm Hg.

We used 45 1-mL plastic samplers (arterial-line-draw blood-sampling kit, Portex, Keene, New Hampshire), with dry lithium heparin, designed for obtaining blood from arterial lines, and 45 0.24-mL glass samplers (Roche Diagnostics, Indianapolis, Indiana), with dry sodium and lithium heparin, designed for arterial sampling, to obtain a total of 90 arterialized blood samples. By rolling a cube marked with numerals 1 through 6, we randomly assigned each sample to one of 6 groups, until there were 15 samples in each group. Forty-five samples were drawn into plastic samplers; of these 15 were analyzed immediately, 15 were stored at room temperature (22°C) for 30 min before analysis, and 15 were stored in ice water (at 0–4°C) for 30 min before analysis. Forty-five samples were drawn into glass samplers; of these 15 were analyzed immediately, 15 were stored at room temperature (22°C) for 30 min before analysis, and 15 were stored in ice water (at 0–4°C) for 30 min before analysis.

Before analysis, the stored samples were thoroughly mixed for 15 seconds, by hand-rolling horizontally. We measured  $P_{CO_2}$ ,  $P_{O_2}$ , pH, and oxygen saturation using blood gas analyzers (Rapidlab 860, Bayer Healthcare, Diagnostics Division, Tarrytown, New York), which were auto-calibrated and control-checked. The analyzers were in a CLIA (Clinical Laboratory, Improvement Amendments of 1988) certified laboratory, and compliant with all current College of American Pathologists standards for calibration, maintenance, and quality-assurance of blood-gas analyzers and CO-oximeters.

The data were analyzed with statistics software (SPSS 13.0 for Windows, SPSS, Chicago, Illinois), using one-way analysis of variance with repeated measures. Differences were considered statistically significant when  $p < 0.05$ . We applied Tukey's Honest Significant Difference post-hoc analysis to identify statistically significant differences between the standard clinical practice (collecting arterial blood in plastic and analyzing the sample immediately) and the 5 alternative methods.

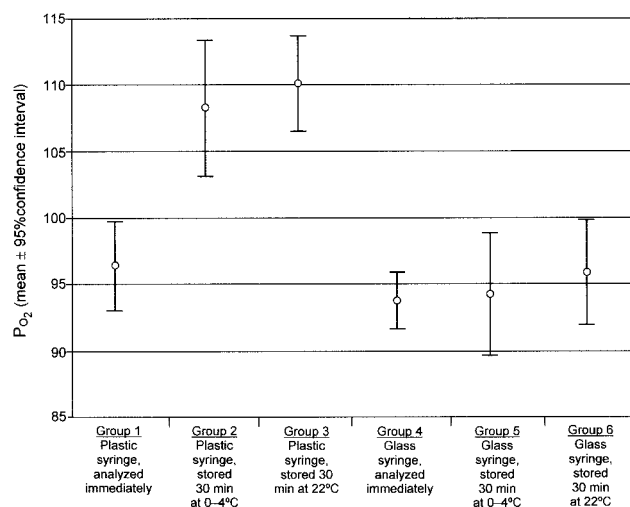
### Results

Table 1 presents the data for  $P_{CO_2}$ ,  $P_{O_2}$ , pH, and oxygen saturation for the 6 groups. There were no statistically significant differences in pH or oxygen saturation among the 6 groups. Compared to the standard clinical practice of collecting arterial blood in plastic and analyzing immediately, there were no statistically significant differences in  $P_{O_2}$  when obtained in glass, analyzed immediately or stored for 30 min, at 22°C or 0–4°C (Fig. 1). However, the  $P_{O_2}$  significantly increased in plastic syringes stored for 30

Table 1. Arterialized Blood Hemoximetry Results for the 6 Pre-Analytical Conditions

Group*	Syringe Type, Time Until Analysis	pH	P <sub>CO</sub> <sub>2</sub> (mean ± SD mm Hg)	P <sub>O</sub> <sub>2</sub> (mean ± SD mm Hg)	Oxygen Saturation (mean ± SD %)
1	Plastic syringe, analyzed immediately	7.36 ± 0.31	35.7 ± 6.4	96.4 ± 6.0	97 ± 0.36
2	Plastic syringe, stored 30 min at 0–4°C	7.34 ± 0.18	36.1 ± 0.71	108.3 ± 9.2†	97 ± 0.39
3	Plastic syringe, stored 30 min at 22°C	7.34 ± 0.018	36.4 ± 0.69	110.1 ± 6.5†	97 ± 0.39
4	Glass syringe, analyzed immediately	7.38 ± 0.021	34.8 ± 1.1	93.7 ± 3.9	97 ± 0.62
5	Glass syringe, stored 30 min at 0–4°C	7.36 ± 0.023	34.2 ± 1.2†	94.2 ± 8.3	97 ± 0.71
6	Glass syringe, stored 30 min at 22°C	7.37 ± 0.024	34.8 ± 1.7	95.9 ± 7.1	97 ± 1.0

\*15 samples in each group  
†p < 0.05

Fig. 1. P<sub>O</sub><sub>2</sub> values from samples of arterialized blood, with 6 different sampling/storage pre-analytical conditions.

min at 22°C and 0–4°C. When stored in plastic syringes for 30 min at 22°C, the P<sub>O</sub><sub>2</sub> increased 11.9 mm Hg. When stored in plastic syringes for 30 min at 0–4°C, the P<sub>O</sub><sub>2</sub> increased 13.7 mm Hg. Compared to analyzing immediately or storing in a glass syringe, these are clinically important differences. There was one statistically significant difference for P<sub>CO</sub><sub>2</sub>. P<sub>CO</sub><sub>2</sub> decreased significantly in glass syringes stored for 30 min at 0–4°C, but the decrease (1.5 mm Hg) was not clinically important.

### Discussion

There are several standards of practice for arterial-blood sampling and analysis. These standards are based on sev-

eral previous studies of sample-storage time, storage temperature, and syringe material. One guideline indicates that specimens should be immediately chilled, or analyzed within 10–15 min if held at room temperature, but iced samples can be analyzed within 1 hour.<sup>1</sup> In another guideline, analyzing a specimen stored in a plastic syringe at room temperature for longer than 30 min is contraindicated.<sup>2</sup> The Clinical and Laboratory Standards Institute recommends that samples in plastic syringes should not be iced, but instead kept at room temperature and analyzed within 30 min after collection, and that glass syringes should be used when analysis will be delayed for longer than 30 min.<sup>3</sup> We designed this study to test the 30-min time limit and to avoid some limitations we saw in reports of previous studies. Unlike most other studies, we used only commercially available blood-collection devices with dry anticoagulants designed specifically for obtaining arterial blood. We used the glass Roche arterial micro-sampler in this study, in lieu of glass syringes. d'Ortho studied these devices and found no significant differences in blood-gas values between the glass Roche micro-sampler and glass syringes with P<sub>O</sub><sub>2</sub> values of 650, 400, 200, 130, and 80 mm Hg, and with storage times up to 30 min and storage at 4°C and ambient temperature.<sup>12</sup> We collected and analyzed all samples independently, thereby avoiding all repeat analyses of leftover blood samples, which occurred in other studies. Our arterialization device was in proximity to the blood-gas analyzer, which allowed us to analyze the samples immediately, whereas, the initial analysis performed in other studies was often delayed by 5 min. We simulated drawing blood directly from an arterial line, using a laboratory model, and we included 2 levels of the 3 variables under study. We placed samples in ice water, applying the technique that Harsten et al found

more effective.<sup>13</sup> We used fresh, whole blood and performed blood-gas analysis and hemoximetry simultaneously on a single integrated system.

Our results are similar to those of Mahoney et al, who used 6%, 10%, and 14% O<sub>2</sub> and tonometered whole blood to study the effects of storing samples for 30 min in plastic and glass syringes at 0°C and in plastic syringes at room temperature (22–23°C).<sup>6</sup> Similar to our findings, when arterial blood with P<sub>O<sub>2</sub></sub> values in the normal range was stored in plastic for 30 min, Mahoney et al also found significant P<sub>O<sub>2</sub></sub> increases when the samples were iced (8.4 mm Hg increase) and when they were stored at room temperature (2.6 mm Hg increase). Mahoney et al did not report results for blood stored in glass for 30 min at room temperature.

Liss and Payne studied the stability of blood gases in plastic syringes, in ice and at room temperature, using samples from 75 hospitalized patients.<sup>7</sup> Some samples were iced during transport to the laboratory, whereas others were analyzed immediately. After initial analysis the samples were randomly assigned to be stored either at room temperature or in ice. Blood left over from initial analyses was repeatedly analyzed at 15 min and 30 min. Although the magnitude of the differences was less than that observed by us and by Mahoney et al, Liss and Payne found significant P<sub>aO<sub>2</sub></sub> increases in samples stored for 30 min at room temperature (2.4 mm Hg increase) and on ice (3.0 mm Hg increase).

Schmidt and Muller-Plathe also studied the stability of normal blood-gas values in blood stored in plastic and glass syringes, at 4°C and 22°C, and found similar results.<sup>8</sup>

Beaulieu et al found decreases in P<sub>aO<sub>2</sub></sub> when plastic syringes were stored at room temperature for 30 min, and increases when plastic syringes were stored in ice for 30 min.<sup>9</sup> Although there were no differences initially, some samples had initial P<sub>O<sub>2</sub></sub> values greater than ambient (150–250 mm Hg), which makes it difficult to interpret these findings. Beaulieu et al used a separate hemoximeter to measure oxygen saturation of blood stored in plastic at room temperature and at 0–4°C, and to calculate the arterial oxygen content. Values of percent oxygen saturation were not reported by Beaulieu et al, and it is not described that the arterial-oxygen-content values included the dissolved component of P<sub>aO<sub>2</sub></sub>, measured by a separate arterial blood-gas analyzer, which makes interpreting these findings also difficult.

Smeenk et al studied the variables of syringe material, storage temperature, and time, using samples from 10 patients who were receiving 100% oxygen.<sup>10</sup> The initial analyses were delayed up to 5 min because of the time required to transport the samples to the laboratory, and multiple repeat analyses of blood left in the syringes were performed at 15, 30, 60, and 120 min after the samples were randomly assigned to be stored at room temperature or in an ice bath. Considering the initial P<sub>aO<sub>2</sub></sub> of 500 mm Hg, the

delay of initial analysis, and the multiple repeat analyses of samples, Smeenk et al found that in plastic syringes there was a statistically significant P<sub>O<sub>2</sub></sub> decrease after 30 min in an ice bath (11.2 mm Hg) and at room temperature (40.5 mm Hg). In glass syringes there was no significant difference in P<sub>aO<sub>2</sub></sub> at 30 min when stored in an ice bath, but there was a statistically significant average decrease of 24.7 mm Hg when left at room temperature.

Pretto and Rochford also studied changes in blood that had high P<sub>O<sub>2</sub></sub>. They reported a P<sub>O<sub>2</sub></sub> decrease of 9.1-mm-Hg/min of delay when blood was stored in plastic at room temperature, a 3.7-mm-Hg/min decrease when blood was stored in glass at room temperature, and a 1.4-mm-Hg/min decrease when blood was stored in plastic and iced.<sup>11</sup>

There are several theories to explain the changes observed in arterial blood gas measurements of samples not analyzed immediately and stored at temperatures less than 37°C in different types of plastic and glass. Several physical and physiological events are probably occurring. According to Henry's law of solubility, the solubility of oxygen and carbon dioxide increases, which decreases the gas partial pressure, and, according to Gay-Lussac's law, the partial pressures of oxygen and carbon dioxide decrease with decreasing temperature.<sup>14</sup> According to hemoglobin physiology, the P<sub>50</sub> (the P<sub>O<sub>2</sub></sub> at which hemoglobin is 50% saturated) decreases, which indicates an increase in oxyhemoglobin affinity and a left-shift of the oxyhemoglobin disassociation curve.<sup>15</sup> Metabolism of the blood is thought to be reduced, so there is less oxygen consumption and carbon dioxide production. When the blood is reheated to 37°C for analysis, these processes are thought to reverse without a net change in values. A common theory is that blood metabolism causes the change, which would support icing the samples to stop metabolism of erythrocytes and leukocytes.

When blood is stored in glass for up to 30 min at room temperature, there are no significant changes in P<sub>aO<sub>2</sub></sub> or P<sub>CO<sub>2</sub></sub>; cooling blood from 37°C to 22°C may effectively decrease metabolism. When blood is stored in plastic, changes opposite and inconsistent with metabolism occur, which supports the theory of diffusion of gases through the plastic. This theory posits that when plastic is cooled to 0–4°C, the plastic molecules contract, opening larger pores for oxygen to diffuse through, but not large enough for the larger carbon dioxide molecule to diffuse through. We found no change in oxygen saturation, probably because our P<sub>O<sub>2</sub></sub> values were too high and the changes in P<sub>O<sub>2</sub></sub> were not large enough to effect changes in oxygen saturation at the plateau of the oxyhemoglobin disassociation curve. The theory of diffusion explains our findings.

There is a consensus in the literature that P<sub>O<sub>2</sub></sub> in blood stored in plastic syringes changes significantly over time, and the majority of studies show the greatest change when the blood is stored at 0–4°C. P<sub>O<sub>2</sub></sub> increased in the studies



in which the sampled blood's oxygen partial pressure was less than ambient. The magnitude of the  $P_{O_2}$  increase is clinically important and probably affects patient-care decisions. The  $P_{O_2}$  increase we observed was greater than that reported in other studies, perhaps because our initial blood analysis was performed immediately, and the initial value was smaller and the difference larger. With a patient receiving supplemental  $O_2$  and with the sample stored for 30 min in a plastic syringe, the laboratory would return a falsely high  $P_{O_2}$  value, which could lead to an erroneous decision to decrease the fraction of inspired oxygen, which could lead to hypoxemia. For hypoxemic patients breathing room air, falsely high  $P_{O_2}$  measurements could deny them indicated supplemental oxygen. Compared to studies that used lower  $O_2$  concentrations, when the initial  $P_{O_2}$  values are higher than ambient, the gas-diffusion gradient is reversed and the  $P_{O_2}$  is decreased. Calculations of oxygenation indices, such as percent physiologic shunt and oxygen-exchange index (alveolar-arterial oxygen difference), may be in error and mislead caregivers on the oxygenation status of a patient receiving a high fraction of inspired oxygen.

### Limitations

There are limitations to this study. Although the samples were mixed for at least 15 seconds and visually inspected for homogeneity, the mixing time may have been inadequate. The small syringe and tube sizes may have discouraged cells and plasma from separating during the 30 min of storage prior to analysis, but inadequate mixing may have influenced the oxygen-saturation measurement. We did not study at what point during the 30-min storage interval the  $P_{O_2}$  changes occurred; the change may have occurred steadily over the 30-min period, or the change may have been concentrated at some part of the storage period. Also, we considered only the effects on normal blood gases with partial-pressure differences between blood and atmosphere of approximately 60 mm Hg for  $P_{O_2}$  and 37 mm Hg for  $P_{CO_2}$ . There may be different effects on hypoxemic and hypercarbic specimens.

### Conclusions

To obtain accurate  $P_{aO_2}$  results, samples drawn in plastic syringes should be analyzed immediately. On-ice storage of plastic syringes that contain arterial blood samples is not recommended. If delay of analysis is predicted, the samples should be drawn and stored in glass. In the presence of leukocytosis, the samples should be drawn in glass and stored at 0–4°C.

To further investigate the effects of syringe material, sample-storage time, and temperature on blood gases and oxygen saturation in arterial blood samples, future research should target blood-gas values consistent with respiratory acidosis and mild hypoxemia (as seen with many patients with cardiopulmonary problems) and values on the slope of the oxyhemoglobin disassociation curve.

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