Current Analytical Approaches to Measuring Blood Analytes

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In recent years, laboratory testing in the critical-care setting has increased, a trend due, in part, to the evolution of electrochemical sensors. Various innovations have extended sensor lifetimes, reduced sensor maintenance, and led to the development of single-use and unit-use disposable sensors. These sensor technologies allow the accurate and precise determination, either at or near the bedside, of several analytes including P_O2, P_CO2, pH, Na, K, Cl, ionized calcium, hematocrit, total hemoglobin, and glucose. Use of these new systems, however, has raised new issues regarding sensor calibration and sample handling and collection. The number of direct-reading analyzers for electrolyte determinations has also increased dramatically. Issues regarding calibration of ion-selective electrodes (ISEs) for Na/K have also been raised after demonstrations of between-instrument variation. Recently, collaborative efforts between eight ISE instrument manufacturers and the National Institute of Standards and Technology resulted in the development of a Standard Reference Material, SRM 956, for the purpose of standardizing direct-reading Na/K ISEs to the flame photometer. Other widely used technologies that provide noninvasive, continuous monitoring include pulse oximetry and transcutaneous gas electrodes. These trends are expected to continue and to produce a new generation of electrochemical and optical sensors.

Additional Keyphrases: ion-selective electrodes · reference materials · calibration · electrochemical sensors · electrolytes · pulse oximetry · transcutaneous gas electrodes

In recent years, laboratory testing in the critical-care setting has increased, both in the numbers and variety of tests offered, and also in the types of individual performing the tests. The acute-care or "stat" laboratory has evolved from individual blood gas analyzer stations to an integrated, multidisciplinary laboratory offering testing for surgical procedures and for emergency room and intensive-care-unit patients. The development of these laboratories has paralleled the technological advances in both laboratory equipment and medical care. In a study reported in 1984, Hall and Shapiro (1) surveyed 227 acute-care/blood gas laboratories with regard to operational characteristics, personnel, analyses performed, and quality-assurance procedures. All of the laboratories performed blood gas/pH analyses and many also determined associated analyses, such as oxyhemoglobin, oxygen saturation, and hematocrit/hemoglobin concentration; 18% also provided one or more tests that are not directly associated with respiration, e.g., determinations of sodium/potassium, calcium, osmometry, and glucose. Of the 227 laboratories, only 130 (67%) used appropriate quality-control procedures for blood gas/pH. A greater percentage (84–100%) were performing acceptable quality-control procedures for the other analytes measured. Burrin and Fyffe (2) surveyed 14 clinical biochemistry laboratories in the Northwest Thames region in England with regard to instrumentation, locations in the hospital, users, and quality assurance. Of the 22 blood gas analyzers they found in use outside the clinical laboratory, most were located in intensive-care units or special-care pediatric units and were mainly used by the anesthesia staff or medical personnel. Little laboratory training had been given to any of the users of these instruments, despite the fact that the clinical laboratory was responsible for maintenance of 12 of the analyzers. For 21 of the instruments, acceptable internal quality-control procedures were used, but none was enrolled in any external quality-assessment scheme.

Blood Gas/Electrolytes

This trend in increased testing outside the traditional laboratory and the emergence of the acute-care laboratory is due, in part, to the evolution of electrochemical sensors over the past three decades. These innovations have led to extended sensor lifetimes, reduced sensor maintenance, the development of single-use disposable sensors, and the use of small sample volumes. Today's sensor technologies allow the accurate and precise determination of several analytes, either at or near the bedside. The introduction of the combination of blood gas/electrolyte analyzers allows rapid, direct analyses of various analytes simultaneously in whole blood, reducing response time to 1–2 min. The multichannel systems are available from several companies, including Nova Biomedical (Waltham, MA), Ciba Corning (Medfield, MA), Instrumentation Laboratory (Lexington, MA), and Mallinckrodt Sensors (Ann Arbor, MI), and perform various combinations of tests, e.g., for pH, P_CO2, P_O2, sodium (Na), potassium (K), chloride (Cl), ionized calcium (Ca2+), hematocrit, total hemoglobin, and glucose. The first three manufacturers design equipment that is best suited for a laboratory setting, whereas the last one produces an instrument (Gem-Stat) suitable for the ward or possibly the bedside. In Gem-Stat, exemplifying a unit-format, the calibrators, sensors, and waste container are prepackaged in a cartridge that can be used for 48 h to analyze as many as 50 individual specimens.

The appearance of these combination systems has raised a new issue regarding calibration of the ion-selective electrodes (ISEs) used in these analyzers. The phosphate buffers developed at the National Institute of Standards and Technology (NIST) and used in most stand-alone blood gas systems are not acceptable for calibration in the multichannel analyzers in which pH and electrolytes are calibrated simultaneously (3). Binding of Na, K, and Ca2+ by the phosphate buffers reduces the Na, K, and Ca2+ ions.

1 Nonstandard abbreviations: ISE, ion-selective electrode; NIST, National Institute of Standards and Technology; NCCCLS, National Committee for Clinical Laboratory Standards; and SRM, Standard Reference Material.
activity coefficients. Use of the zwitterionic buffers, 3-(N-morpholino)propanesulfonic acid and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (MOPS and HEPES, respectively) to eliminate this problem is necessary for simultaneous calibration of pH and electrolytes. Moreover, the interchange of calibrating solutions between blood gas analyzers and multichannel analyzers even from the same manufacturer may not be possible, and may cause erroneous results.

Use of these systems also necessitates a review of sample storage criteria and choice of anticoagulant. Misiano and Moran (4, 5) recently addressed the issue of sample transport and storage in relation to potassium values. Although storage in an ice-water slurry is considered the ideal for blood gas determinations, it has adverse effects on potassium determinations. In their study, clinical specimens were sent un-iced to the laboratory and assayed. The excess specimens were divided into two equal aliquots: one was stored at room temperature, the other in crushed ice. The specimens were then analyzed at timed intervals, from 25 to 80 min later. As has been reported previously (6, 7), storing whole-blood specimens on ice before measurement increases potassium values by amounts that are both clinically (0.14–0.40 mmol/L) and statistically significant (P <0.001 at all times measured). Individual specimens yielded differences in excess of 1.5 mmol/L. Other factors, such as instrumentation and interindividual variations, also contributed. The presence of cold agglutinins in a sample that is iced before analysis may increase potassium by one- or twofold, depending on how long the sample is in the ice.

Although the effect of excess lithium and sodium heparin on ionized calcium values has been reported previously (8–10), its impact has become more widespread as ionized calcium sensors are added to combination blood gas/electrolyte systems. The commonly used 3-mL pre-packaged syringes for blood gas determination contain between 75 and 200 USP units of heparin; filled, the syringes contain about 25–66 USP units of heparin per milliliter of blood. Urban et al. (9) suggest that sodium heparin concentration in excess of 10–15 USP units/mL will cause significant binding of ionized calcium. Calcium-titrated heparin or electrolyte-balanced heparin (available from Radiometer America, Inc., Westlake, OH, and from Ciba Corning) will reduce this effect and can be used in concentrations up to 50 int. units/mL (11). Studies of paired samples in our laboratory compared ionized calcium values in 3-mL syringes containing dry sodium heparin at 25 USP units/mL vs liquid calcium-titrated heparin at about 30 int. units/mL. Use of sodium heparin gave a consistent decrease in ionized calcium of 0.1 mmol/L (Table 1).

The number of direct-reading sensors for determination of electrolytes, including lithium, has also increased dramatically. These include traditional electrochemical sensors, as well as single-use disposable sensors. Among the latter are the Kodak DP60 and E700 (Eastman Kodak, Rochester, NY) and the ChemPro (Johnson & Johnson Professional Diagnostics, Roseville, MN). The Kodak equipment can assay serum or plasma; the ChemPro is capable of assaying whole blood, serum, or plasma. Both systems use a multilayer approach, in which the traditional electrochemical sensors are placed on planar substrates. As this and other similar technologies develop, we anticipate several improvements, including multi-sensor arrays on single slides or cards, improved uniformity and reliability, and low cost.

Calibration of the ISEs for electrolyte measurements has been a controversial topic for many years. Most manufacturers recommend using their own calibration standards and control material, materials that range from simple aqueous solutions to complex mixtures containing bovine serum albumin. Given the differences in calibration approaches, it is not surprising that these instruments give different electrolyte values for the same sample of human serum. Gunaratna et al. (12) have shown that ISE-based instruments from different manufacturers give results for Na/K determinations that differ by 2–5%. In 1985, a research project sponsored by the National Committee for Clinical Laboratory Standards (NCCLS) and funded by eight instrument manufacturers (Amdev, AVL, Ciba Corning, Eastman Kodak, Instrumentation Laboratory, Kone, Nova, and Radiometer) was undertaken at NIST. The goal of the project was to develop standard reference materials to be used by manufacturers and laboratories to bring conformity to direct potentiometric measurements of Na/K (12, 13). A series of four interlaboratory round-robin tests was performed by the eight manufacturers on their equipment and by four hospital laboratories. In each round-robin protocol, between three and 11 candidate reference materials, including aqueous standards, standards containing bovine serum albumin, and human-serum-based materials, including variations of NIST Standard Reference Material 909, were assayed. The results indicated that use of an ultrafiltered human serum pool (at three concentrations) as a standard material for calibrating direct potentiometric ISEs would bring conformity among instruments from various manufacturers. After post-calibration with this material, interlaboratory precision was improved by two-to threefold for both Na and K. The new Standard Reference Material, designated SRM 956, should be available in 1990. Sodium and potassium values will be determined by both Definitive and Reference Methods at NIST. It is hoped that future efforts in this area will lead to development of

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<th>Heparin type*</th>
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* Calcium-titrated heparin was in liquid form (Radiometer, Copenhagen, Denmark); its approximate concentration in blood was 25–30 int. units/mL. Sodium heparin was in dry form in prepackaged syringes (Sherwood Medical, St. Louis, MO); its approximate concentration in a filled syringe was 25 USP units/mL.

** Calcium ionized
similar Standard Reference Materials for ionized calcium analyzers.

A recently published NCCLS Proposed Standard (C29-P) recommends standardization of direct ISEs (those that do not require predilution of the specimen) for Na/K to the flame photometric reference method (14). It further recommends use of SRM 956 as the standard material, thus eliminating any variation due to differences in standardization of flame photometers by the manufacturers. Individual laboratories can utilize SRM 956 to verify calibration by manufacturers and also to ensure comparability of results between direct ISEs, indirect ISEs, and flame photometers in use in their institutions.

**Continuous Noninvasive Monitors**

Several technologies have been used clinically to provide noninvasive, continuous monitoring of PaO₂ and PaCO₂. Two of the most prominent are pulse oximetry and transcutaneous gas electrodes (15), both of which make their measurement through the patient's skin and monitor the measurements continuously without requiring withdrawal of a specimen. In addition, miniaturized noninvasive gas sensors have been developed for placement at the inner lining of the eyelid (conjunctival sensors) (16).

The development and clinical utility of transcutaneous measurements (termed PtcO₂ and PtcCO₂) have been well documented (17-20). The sensors for these determinations are the same as their counterparts used in conventional blood gas analyzers, i.e., the Clark-style oxygen and the Stow-Severinghaus-style carbon dioxide electrodes. Both individual and combination electrodes are available (19). In all cases, the sensors are heated at 43-45 °C to produce a stable hyperemic flow of arterialized blood in the dermis and to reduce the diffusion barrier of the epidermis. The net result is oxygen diffusion through the skin in concentrations resembling the PtcO₂ (15).

Although the transcutaneous electrodes are useful as trend monitors, one must recognize that transcutaneous blood gases are not the same as arterial blood gases. Several important factors affect the relationship between the two. First, oxygen is consumed by the skin between the vascular bed and the transcutaneous electrode, which tends to decrease the PtcO₂ measurement and increase the PtcCO₂. Second, the electrode heats the underlying skin, which tends to increase both PtcO₂ and PtcCO₂. For PtcO₂, these two factors nearly cancel one another so that PtcO₂ and PtcCO₂ are similar, particularly in neonates. For PtcCO₂, the two factors are additive, making PtcCO₂ greater than PtcO₂ (21).

The use of transcutaneous gas electrodes has found wide acceptance for neonatal monitoring (20,22), and more recently in adults (23). In most patients with normal cardiac output, PtcO₂ follows the trend in PaO₂ and decreases relative to PaO₂, as the patients' ages increase (24). However, in the presence of severely reduced cardiac output and peripheral perfusion, the PtcO₂ values will deviate from PaO₂ values and become flow dependent, thus providing quantitative information regarding cardiac output (22). This has been tested experimentally in dogs (25) and subsequently confirmed in clinical series (25). Similar divergences between PtcO₂ and PaO₂ have been demonstrated (26). Therefore, a decrease in PtcO₂ may be due to a decrease in PaO₂ or a decrease in cardiac output. Thus, the PtcO₂ is an indicator of tissue oxygen delivery: arterial oxygenation and cardiac output (21). Clinically, this divergence is not an error to be avoided, but rather a highly significant noninvasive indicator of circulatory compromise (22).

Several technical considerations must be observed when these sensors are being used: proper maintenance, membrane preparation and placement, calibration, and proper preparation of the skin. Because of the potential for causing skin burns with the heated electrodes, some guidelines have been developed. A temperature of 43.0 to 43.5 °C is commonly used for premature infants, and the location of the sensor on the skin is changed every 2 h. The electrode temperature and length of time it can be left on the skin can be increased as the patient matures and skin thickness increases. For newborns, a temperature of 43.5 °C for 4 h is safe; for children and adults, 44 °C and 4-8 h is safe (24).

Several reports have evaluated the effect of halothane on the transcutaneous oxygen electrode. Although some reported significant upward drift in the PaO₂ reading (27), another report showed no clinically significant differences (28). Clearly, electrode design and membrane composition are important factors, and each sensor must be evaluated individually.

**Pulse Oximetry**

Another of the continuous, noninvasive technologies in wide use is pulse oximetry, which provides a measurement of arterial oxygen saturation (SaO₂). The pulse oximeter combines the principles of spectrophotometric oximetry and plethysmography and functions by placing a living tissue (usually the ear, foot, toe, hand, or finger) between a two-wavelength light source and photodiode detector. Light is then transmitted through the tissue at the two wavelengths, about 660 and 940 nm, and monitored during each pulse. The degree of change in transmitted light is proportional to the arterial pulse change, wavelength of light used, and oxyhemoglobin saturation. Assuming that the pulsatile waveform is entirely due to the passage of arterial blood, one can calculate SaO₂ continuously (15). Most pulse oximeters also display pulse rate.

Although widely accepted in monitoring neonates, children, and adults, there are limitations to this technology. If perfusion is poor, the pulse oximeter may not be able to adequately differentiate between arterial pulsation and background noise. In addition, the oximeters are subject to motion artifact (21) and to other factors such as hypothermia, vasopressor drugs, and stray ambient lighting (29). Because pulse oximeters use two-wavelength spectrophotometry, they measure only oxyhemoglobin and deoxyhemoglobin and are unable to detect dyshemoglobins. For example, pulse oximetry is blind to the effects of carboxy and methemoglobin and will overestimate the true oxygen saturation in the presence of these hemoglobin derivatives. Fetal hemoglobin, however, apparently has little or no adverse effect (21). Severinghaus and Naifeh (30) assessed the ability of six different pulse oximeters to accurately measure low oxygen saturation (SaO₂ <70%). They recorded SaO₂ during rapidly induced plateaus of profound hypoxia (40-70% saturation) in normal volunteers. They not only found between-instrument variation, but also determined that some instruments displayed near normal saturation when the in vitro measurement gave values in the range of 40-70%. Thus, the performance of pulse oximeters below 70% saturation remains marginal at best. Finally, both total hemoglobin and the presence of dyshemoglobins must be assessed before calculating oxygen content from a measurement of SaO₂ by pulse oximetry (SaO₂).

When used in the proper context, pulse oximetry offers
many advantages in the continuous monitoring of critically ill patients: rapid and simple setup, no warm-up time, no calibration, no sensor heating, and no hourly maintenance. Excellent correlations with results from multiwavelength co-oximeters have been reported in very low-birth-weight infants (31), neonates (32–34), children, and adults (35–37). In most cases, the study populations had $s_{\text{AO$_2$}}$ values between 75% and 100%. Several studies have found pulse oximetry to be an appropriate alternative to $p_{\text{AO$_2$}}$ (31–33), particularly in situations of poor peripheral perfusion. In addition, saturation may be a more sensitive indicator of oxygenation than is $p_{\text{AO$_2$}}$ in infants with relative hypoxemia, owing to the shape of the oxyhemoglobin dissociation curve. On the steep portion of the curve, small changes in $p_{\text{AO$_2$}}$ are associated with marked saturation changes (38).

At the other extreme (the flat portion of the curve), large increases in $p_{\text{AO$_2$}}$ may be associated with only small changes in $s_{\text{AO$_2$}}$, so that saturation may be a less sensitive index of oxygenation. Therefore, in acutely ill infants who may be hyperoxicemic, great caution must be exercised in predicting $p_{\text{AO$_2$}}$ from $s_{\text{AO$_2$}}$. Walsh et al. (38) assessed the ability of pulse oximetry to predict $p_{\text{AO$_2$}}$ in two groups of infants, one with chronic lung disease and the other with acute cardiopulmonary disease. In the infants with chronic lung disease, $p_{\text{AO$_2$}}$ derived from pulse oximetry was within 10 mmHg of measured $p_{\text{AO$_2$}}$ in 73% of comparisons; in the acute group, calculated $p_{\text{AO$_2$}}$ was within 10 mmHg of measured $p_{\text{AO$_2$}}$ in only 50% of comparisons. Correcting the oxygen dissociation curves for the concentration of hemoglobin in the acute infants did not change the results. The authors concluded that $s_{\text{AO$_2$}}$ and derived $p_{\text{AO$_2$}}$ are useful in infants with chronic lung disease, but emphasized that full awareness of the limitations is important. In infants with acute cardiopulmonary problems, pulse oximetry unreliably reflects $p_{\text{AO$_2$}}$, but may be useful in detecting clinical deterioration.

Pulse oximetry has proven very useful for the continuous monitoring of oxygenation. However, no data are available to support the use of pulse oximetry as a replacement for arterial blood gases. When clinical conditions do not agree with pulse oximetry readings, arterial analysis is warranted (15).

Additional Testing Methods

Reflectance meters for bedside glucose testing have been used extensively for both emergency and routine monitoring as well as in the home. Several published reports have documented their successful use (39–41), but a few have reported poor correlation between bedside determination of glucose and the quantitative laboratory procedure (42, 43). Clearly, these techniques are operator-dependent and require careful training and monitoring to be successful. Most glucose meters utilize strips that incorporate the well-established glucose oxidase/peroxidase chemistry. In a recently developed glucose meter system, The ExactTech (MediSense, Cambridge, MA), the strip incorporates an electron transfer mediator, ferrocene, which takes the place of oxygen in the reaction of glucose oxidase with glucose and is detected amperometrically. This system is similar to the single-use disposable electrodes previously discussed.

Lactate concentration in whole blood can also be determined by several analyzers available from YSI Inc. (Yellow Springs, OH). These systems utilize an enzyme electrode technology in which the enzyme L-lactate oxidase is immobilized in a thin membrane and placed over the sensor. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being determined amperometrically. The longest response time of these systems, from sampling aspiration to result, is 1 min.

In summary, it is clear that rapid development of new sensors and improvement of existing sensors will continue.

The evaluation of single-use and unit-use disposable sensors has brought critical-care testing closer to the patient’s bedside. The combination multichannel blood gas/electrolyte systems have allowed us to offer an array of critical analyses simultaneously, rapidly, and with enhanced precision. In the future, we will undoubtedly see a continuation of these trends and the evolution of a new generation of electrochemical and optical sensors.

References

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