Effects of Icing Whole-Blood Samples in Plastic Go Beyond $P_{O_2}$ Alone

To the Editor:

I read with interest the Letter of Ooi and Tokessy (1) describing their study of the effects on $P_{O_2}$ of icing of blood-gas samples in plastic (polypropylene) syringes. As they correctly cite, Mahoney et al. (2) reported earlier in this journal this phenomenon of increased $P_{O_2}$ after iced plastic syringe storage, and this has been observed in informal studies as well.

Ooi and Tokessy also note that the National Committee for Clinical Laboratory Standards (NCCLS) document on Blood Gas Pre-Analytical Considerations, C27-T (3), does not agree with the Mahoney et al. findings, or their own. As current chair of the NCCLS working group responsible for developing document C32-P, which addresses the issues relative to this subject, and a member of the NCCLS subcommittee on Blood Gas, I can point out that the present "tentative" (not "proposed," as stated version of the NCCLS document is in the final stages of the NCCLS consensus process to be upgraded to an approved level document and now includes the issues discussed in the paper of Mahoney et al.

Two problems associated with icing have not received the same attention as the $P_{O_2}$ issue, but may be more important clinically. Both issues relate to measurements of hemoglobin and electrolytes in the same sample as used for blood gas measurements, in a common analyzer—a frequent practice, especially in emergency environments.

For total hemoglobin measurements, the basic requirement for mixing the sample adequately is more difficult to accomplish in an iced sample of blood contained in a syringe. This is the result of both the lack (one hopes) of air space in the syringe, which in the evacuated sample tube facilitates mixing, as well as the increased viscosity of the whole-blood sample because of the cold temperature. The practical experience of many laboratorians frequently exacerbates this issue, because mixing a syringe sample that contains blood for gas and pH analysis alone shows little effect on the "blood gas only" values. The result of this failure to adequately mix can be widely discrepant values for hemoglobin on the same patient when measured on the "blood gas plus" analyzer at different times or compared with hematology-obtained values.

The second issue is the measurement of potassium in the same sample that has been iced. As the sample is cooled, potassium is released from the cells, yielding a subsequent increase in its measured concentrations (4, 5). Although not typically very significant clinically, these potassium increases can, in individual patients, amount to several millimoles per liter. However, one recent in vitro experiment with erythrocytes suspended in buffer did not report the same effect (6). Anecdotal information relates this dramatic increase to the presence of cold agglutinins or extremely high leukocyte or thrombocyte counts.

Although the Ooi and Tokessy information certainly is a good supplement to the thorough study by Mahoney et al., I would be concerned about a blanket recommendation to continue icing the blood unless these other factors are taken into account.

References
4. Moran RF, Grenier RE. Effects of "standard" blood gas transport and storage conditions on electrolyte results with observations on reported hemoglobin measurement anomalies. In: Methodology and clinical applications of ion-selective electrodes, Vol. 10. IPCP Technical Secretariat, 1989.

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Avoid DNA/RNA Contamination in Polymerase Chain Reactions

To the Editor:

We support and concur with the recent warning by Reyes et al. (1) concerning potential artifacts associated with trace genomic DNA contamination in RNA samples used for coupled reverse transcriptase/polymerase chain reaction (PCR) amplification to monitor gene expression. For a gene that contains introns, oligonucleotide primers can be selected that flank an intron, and the PCR-amplified products deriving from cellular RNA or contaminating genomic DNA can be distinguished on the basis of size. However, when the intron/exon structure of the gene of interest is unknown or lacks introns (see Danciger et al. (2) for a brief listing), contamination of RNA with genomic DNA can be a source of artifacts. In a recent report describing our studies characterizing the tissue expression of such an intronless gene (3), we addressed these concerns and provided appropriate protocols for DNase pretreatment of the RNA, a test to ensure that the DNase is truly RNase-free, and a demonstration of the importance of several negative controls. Finally, it is essential to ensure that the added DNase be totally and irreversibly inactivated before the PCR to prevent loss of the final PCR product (4). Another approach to deal with these concerns has been reported by Schuldiner et al. (5).

References