MINIMUM VOLUME OF DISCARD FOR VALID BLOOD SAMPLING FROM INDWELLING ARTERIAL CANNULAE

M. C. C. CLAPHAM, N. WILLIS AND W. W. MAPLESON

Indwelling arterial cannulae are used widely in the intensive care unit to permit pressure monitoring and blood sampling. The patency of the cannulae and connecting tubing is maintained by flushing with heparinized saline. Before taking samples for analysis, the heparinized saline in the catheter must be discarded to avoid dilution. Personal observation showed that, in the intensive care unit in the University Hospital of Wales, the volume of discard varied, between clinicians, from 3 to 10 ml. Palermo, Andrews and Ellison (1980) have studied this problem in relation to the assessment of coagulation; Dennis and colleagues (1985) have studied it in relation to the measurement of blood-gas tensions, but only for a catheter of unspecified internal volume.

In order to ascertain the minimum discard volume needed to obtain a representative arterial blood sample, some relevant biochemical indices were analysed in the first 10 ml of fluid taken from arterial catheters in five 2-ml increments.

SUMMARY

The volume of discard for valid blood sampling from indwelling femoral (leader cath) and radial (Venflon) arterial cannulae (with internal volume of cannula plus connecting tubing of approximately 2 ml) was investigated by analysing sequential 2-ml aliquots of 10-ml samples. The aliquots were analysed for pH, carbon dioxide tension, oxygen tension, standard bicarbonate, haemoglobin concentration, haemoglobin oxygen saturation and potassium concentration. Analysis of variance showed that, for these variables and these catheters, a valid blood sample was obtained after discarding 4 ml, but not after only 2 ml. The haemoglobin concentration, as measured by a Corning oximeter, provided good warning of inadequate discard.

Viggo Products, 4614-4, volume 0.1 ml) via 200-cm connecting tubing (lectro-cath, Vygon, 1155.20, volume 1.85 ml). The total volume of both systems was close to 2.1 ml. The volumes were determined by weighing each component, and each complete system, dry and full of water.

In each patient, each system was sampled on three separate occasions. On each occasion the sample consisted of five successive aliquots, each drawn into a separate 2-ml polypropylene syringe (Monoject). The syringes were heparinized using 0.5 ml of sodium heparin, 1000 u ml⁻¹, which was then discarded leaving only that volume (~0.05 ml) which remained in the hub of the syringe. All samples were taken by the same person (M.C.) to ensure that the technique and rate of sampling (about 10 s for 2 ml) were consistent.

Each aliquot was analysed in duplicate using a Corning 178 blood-gas analyser and Corning oximeter M2500 (Ciba Corning Ltd, Halstead, Essex) for: pH, carbon dioxide tension (Pco₂),

M. C. C. CLAPHAM,* M.B., B.S., F.F.A.R.C.S. (Department of Anaesthetics); N. WILLIS, F.I.M.L.S. (Department of Medical Biochemistry); W. W. MAPLESON, D.SC., F.INST.P. (Department of Anaesthetics); University of Wales College of Medicine, University Hospital of Wales, Heath Park, Cardiff, CF4 4XW. Accepted for Publication: August 26, 1986.

* Present address: Department of Anaesthetics, East Birmingham Hospital, Bordesley Green East, Birmingham. Correspondence to W. W. M.
DISCARD VOLUME FOR VALID BLOOD SAMPLING

Table I. Means and standard errors of the differences (SED) of various sets of blood measurements on 2-ml aliquots taken sequentially through arterial catheters with approximately 2 ml internal volume. *P < 0.001 with respect to aliquot 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_O_2$ (kPa)</td>
<td>23.6</td>
<td>22.5</td>
<td>22.2</td>
<td>22.3</td>
<td>22.2</td>
<td>1.13</td>
</tr>
<tr>
<td>$S_O_2$ (%)</td>
<td>—</td>
<td>96.4</td>
<td>96.5</td>
<td>96.4</td>
<td>96.4</td>
<td>0.05</td>
</tr>
<tr>
<td>pH</td>
<td>7.39*</td>
<td>7.43</td>
<td>7.42</td>
<td>7.43</td>
<td>7.42</td>
<td>0.005</td>
</tr>
<tr>
<td>$P_Co_2$ (kPa)</td>
<td>0.58*</td>
<td>5.25*</td>
<td>6.00</td>
<td>6.08</td>
<td>6.12</td>
<td>0.13</td>
</tr>
<tr>
<td>StB (mmol litre$^{-1}$)</td>
<td>11.9*</td>
<td>26.6*</td>
<td>29.0</td>
<td>29.2</td>
<td>29.2</td>
<td>0.40</td>
</tr>
<tr>
<td>K$^+$ (mmol litre$^{-1}$)</td>
<td>0.5*</td>
<td>3.5*</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>0.07</td>
</tr>
<tr>
<td>Hb (g dl$^{-1}$)</td>
<td>—</td>
<td>9.7*</td>
<td>10.6</td>
<td>10.6</td>
<td>10.6</td>
<td>0.06</td>
</tr>
</tbody>
</table>

With a slight exception in the case of $P_Co_2$ (see Appendix) there was no significant difference between catheters ($P > 0.1$) and no significant catheter–aliquot interaction ($P > 0.1$). Accordingly, the results for each variable are summarized in table I in terms of the mean, for each aliquot, of both analyses of all samples for both catheters. The differences between means should be interpreted by reference to the standard errors of the differences between the pairs of means, which are given in the last column. The oximeter was unable to read the first aliquot, because the haemoglobin was below the measurement threshold (5 g dl$^{-1}$). For interest, the rate of deterioration of the samples with time is shown in table II.

Table II. Coefficients of the regression equation $\dot{y} = a + bx$, where $\dot{y}$ = rate of change of variable with time (rate per min) and $x$ = mean value of the two analyses (d.f. = 148)

<table>
<thead>
<tr>
<th>Variable</th>
<th>$a$</th>
<th>$b$</th>
<th>SE($b$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.0072</td>
<td>0.00091</td>
<td>0.00113</td>
</tr>
<tr>
<td>$P_Co_2$ (kPa)</td>
<td>-0.0035</td>
<td>0.00109</td>
<td>0.00051</td>
</tr>
<tr>
<td>$P_O_2$ (kPa)</td>
<td>0.333</td>
<td>-0.0156</td>
<td>0.0011</td>
</tr>
<tr>
<td>StB (mmol litre$^{-1}$)</td>
<td>-0.0044</td>
<td>-0.00114</td>
<td>0.00134</td>
</tr>
<tr>
<td>$S_O_2$ (%)</td>
<td>-1.66</td>
<td>0.0172</td>
<td>0.0086</td>
</tr>
<tr>
<td>Hb (g dl$^{-1}$)</td>
<td>-0.0076</td>
<td>0.00045</td>
<td>0.00096</td>
</tr>
</tbody>
</table>

Statistics

With analysis of each batch of aliquots spread over 20–25 min, there was generally some deterioration of the sample with time. This deterioration was estimated for each variable with a linear regression technique (see Appendix) and the estimate used to correct all measurements to what each would have been if it had been made at the time of the first analysis of each batch. An analysis of variance was performed on the corrected measurements. The structure of this analysis (see Appendix) distinguished (1) random variation between patients, between samples, between aliquots and between measurements, and (2) systematic differences between catheters and between aliquots, and any systematic catheter–aliquot interaction. The catheter–aliquot interaction will reveal any tendency for contamination to become insignificant after fewer aliquots with one catheter than with the other.

The analysis of variance was performed using the Genstat statistical package, version 4.03 (Numerical Algorithms Group Ltd) on a Honeywell mainframe computer running under Multics release 10.1.

DISCUSSION

It can be seen that the first aliquot was clearly anomalous for most variables and that the second was also significantly different from the remainder for $P_Co_2$, StB, K$^+$, and Hb.

Although the $P_O_2$ results show no significant difference between the aliquots, it is clear that the oxygen tension ($P_O_2$), standard bicarbonate (StB), haemoglobin concentration (Hb) and haemoglobin oxygen saturation ($S_O_2$). The order in which the specimens were analysed was randomized separately for the initial and duplicate analyses. The potassium concentration (K$^+$) was measured once on the remaining serum using a Beckman ISE electrolyte analyser E2 (Beckman, High Wycombe, Bucks). Blood analysis, on well-mixed samples, was started within 5 min of collection and concluded within 30 min, the samples being kept at room temperature.

RESULTS

With a slight exception in the case of $P_Co_2$ (see Appendix) there was no significant difference between catheters ($P > 0.1$) and no significant catheter–aliquot interaction ($P > 0.1$). Accordingly, the results for each variable are summarized in table I in terms of the mean, for each aliquot, of both analyses of all samples for both catheters. The differences between means should be interpreted by reference to the standard errors of the differences between the pairs of means, which are given in the last column. The oximeter was unable to read the first aliquot, because the haemoglobin was below the measurement threshold (5 g dl$^{-1}$). For interest, the rate of deterioration of the samples with time is shown in table II.
pattern of results mirrored that of $P_{\text{CO}_2}$, StB, and $K^+$. The explanation no doubt lies in the fact that, in this study, the $P_{\text{O}_2}$ of the first aliquot was essentially that of a specimen of heparinized saline equilibrated with air at room temperature and measured at 37 °C, and that the $P_{\text{O}_2}$ values in our patients happened to be similar. If the $P_{\text{O}_2}$ of the blood were more removed from that of the saline, then no doubt there would be significant differences between aliquots.

It would appear that, for the present variables, and for both types and sites of cannula (with a total internal volume of approximately 2 ml), valid results are obtained for all measurements after a 4-ml discard but not after a 2-ml discard. This is in agreement with Palermo, Andrews and Ellison (1980) who recommended a discard volume of twice the internal volume, on the basis of coagulation measurements on blood samples drawn through catheters of 1.5 and 1.8 ml internal volume. A close study of their results suggests that a smaller discard volume might possibly be adequate. On the other hand Dennis and colleagues (1985), measuring blood-gas tensions and spun haematocrit, found that a 20-gauge, 3.2-cm radial arterial cannula, together with a 7-ft (2.1-m) connecting tube required a discard volume of 10 ml before sampling was satisfactory. They did not state the internal volume of their system, but recommended that each intensive care unit should make a similar study to determine the appropriate volume. This may well be sound advice, since it can be inferred from table I that, whatever the internal volume of a catheter system, the minimum discard volume will depend on (1) the extent to which the measurement is altered by the contaminating flushing fluid and (2) the extent of the error in measurement that is acceptable. In addition, it is conceivable that the speed at which the sample is drawn may affect mixing in the catheter and, hence, may influence the result.

With most of the variables in this study, the true value in a patient can change rapidly, often without clinical signs. Therefore, if the measurement of one of these variables changes from one blood sample to the next, it may be impossible, from that measurement alone, to discriminate between a true change and contamination of the sample. However, major changes in haemoglobin concentration are associated with marked clinical signs and symptoms (e.g. acute blood loss) or with clinical intervention (e.g. fluid administration). Accordingly, an unexpectedly low haemoglobin concentration can be an indicator of inadequate discard volume. Therefore, the concurrent, routine determination of haemoglobin concentration in all blood samples for blood-gas and potassium analysis could help identify invalid samples.

Invalidity of the sample is of particular importance in the case of potassium concentration. If this is falsely reported to be low, it is likely that potassium will be administered and this can result in cardiac arrest and even arrest.

**APPENDIX**

**CORRECTION FOR DETERIORATION OF SAMPLE WITH TIME**

The rate of change with time of each variable (except for $K^+$, which was measured only once per aliquot) was estimated for each aliquot according to $\dot{y} = (t_2 - t_1)/(t_2 - t_1)$ where $t_1$ and $t_2$ are the times and $t_1$ and $t_2$ are the results of the two analyses. The rate of change of a variable may vary with its actual value: for example, a low $P_{\text{O}_2}$ will tend to increase towards the $P_{\text{O}_2}$ of air; a high $P_{\text{O}_2}$ will tend to decrease. Therefore, the linear regression equation $\dot{y} = a + bx$, where $x$ is the mean of $t_1$ and $r_2$, is fitted to the values of $\dot{y}$ calculated as above for each variable. The resulting values of $a$ and $b$ are given in table II, together with the standard errors of $b$. From these it follows that the coefficients $b$ (dependence of rate of change with time
on mean value) vary from very highly significant for $P_{O_2}$ (Student's $t = 14$) to non-significant for some other variables. It is also interesting to note that the values of $a$ and $b$ for $P_{O_2}$ indicate that the rate of change of $P_{O_2}$ would be zero at $0.333/0.0156 = 21.3$ kPa, that is, close to the partial pressure of oxygen in the atmosphere.

For consistency, all readings of all variables were corrected to what they were estimated to be at the time of the first analysis of each sample according to: corrected reading = $r - (a + bx) \times t$, where $t$ is the time since the first analysis of a sample.

**ANALYSIS OF VARIANCE**

The analysis-of-variance model used is shown diagrammatically in figure 1. Each individual (corrected) reading is the sum of:

1. The grand mean of all readings: $\bar{X}$
2. The following random (error) variations:
   - Patient stratum: $P_i = \text{deviation of mean of all measurements on ith patient (i = 1 to 5) from grand mean.}$
   - Sample stratum: $S_{ij} = \text{deviation of mean of all measurements on jth sample (j = 1 to 6) (from ith patient) from mean for ith patient (corrected for the systematic effect of the relevant, mth, catheter—see below).}$
   - Aliquot stratum: $A_{ij} = \text{deviation of mean of all measurements on kth aliquot (k = 1 to 5) (of jth sample from ith patient) from mean for jth sample from ith patient (corrected for the systematic effect of the kth aliquot and for the systematic interaction effect between the mth catheter and the kth aliquot—see below).}$
3. The following systematic ("treatment") variations:
   - Catheter effect: $C_m = \text{deviation of mean of all measurements from mth catheter (m = 1 or 2) from grand mean.}$
   - Aliquot effect: $A_k = \text{deviation of mean of all measurements on kth aliquot from grand mean.}$
   - Catheter-aliquot interaction: $CA_{mk} = \text{deviation of mean of all measurements on kth aliquot from mth catheter from (grand mean + C_m + A_k).}$

Plotting residuals against fitted values showed that the former were reasonably normally distributed although, with $P_{CO_2}$, $StB$ and $K^+$, the variance of the residuals was somewhat different for the first aliquot from that for the remainder. However, when the analysis of variance was repeated for these three variables with only the last four aliquots included, the second aliquot was still highly significantly different from the fifth.

**RESULTS FOR $P_{O_2}$**

$P_{O_2}$ was the only variable with any suggestion of a difference between catheters (variance ratio 5.0; d.f. 1, 24; 0.025 $< P < 0.05$) or catheter–aliquot interaction (variance ratio 2.2; d.f. 4, 112; 0.05 $< P < 0.1$). However, table III shows that the difference between catheters was almost the same for all the last four aliquots. The simplest explanation of this is that the true mean $P_{O_2}$ of the 15 samples drawn through the femoral catheter happened, by chance, to be about 0.6 kPa greater than that of the 15 drawn through the radial catheter.

**REFERENCES**
