Measurement of pH , PCO_2 , and standard bicarbonate on samples of capillary blood

R. S. JONES, V. R. KAMATH, AND J. C. KAY

*From Alder Hey Children's Hospital, Liverpool, and the
Department of Child Health, University of Liverpool*

SYNOPSIS A simple technique for the collection and equilibration of capillary samples of blood is described for the measurement of pH , PCO_2 , and standard bicarbonate by the interpolation method. The possible errors of collecting blood in open-ended capillary tubes containing wet heparin are examined. The method has proved to be accurate, providing the technique is strictly adhered to, and has proved especially valuable when frequent estimations have been required.

The use of capillary blood for the measurement of acid-base abnormalities is a great advantage over arterial sampling especially when a series of measurements is required. The measurement of pH on capillary samples of blood is subject to certain technical errors. We have examined the main sources of error and describe here a technique for collection and for equilibration of capillary samples in order to obtain the PCO_2 and standard bicarbonate by the interpolation method (Andersen, Engel, Jørgensen, and Astrup, 1960).

The accuracy of the method has proved adequate for clinical purposes. The cost of the equilibration device is negligible and it can be constructed in a few hours and used with any pH meter having a discrimination of ± 0.005 pH units and an electrode which will accept a sample size of 0.1 ml. of blood.

METHOD

Approximately 0.1 ml. of blood is collected in a glass melting point tube (A. Gallenkamp & Co. Ltd.), 10 cm. long, and with an internal diameter of approximately 1 mm. Tubes are prepared by washing with distilled water and drying. They are not siliconed. A tube is heparinized immediately before use by picking up a drop of heparin (10 mg./ml.: Evans Medical). The heparin is not neutralized because it was found that the pH of the heparin used has a negligible effect on the final blood pH . The drop of heparin is made to traverse the length of the tube in order to wet the internal surface. The surplus is removed by shaking the tube vigorously several times. Further samples of 0.1 ml. are collected in each of two Pasteur pipettes which are approximately 10 cm. long, the capillary portion being 4-5 cm. in length and the bore approximately 1 mm. They are prepared by washing with

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distilled water and drying. They are not siliconed but are heparinized by the technique described for the capillary tubes.

Figure 1 shows the apparatus used for equilibration. Accurately measured concentrations of CO_2 in O_2 of approximately 4% and 8% strength are passed into the glass humidification chambers 'a' which contain distilled water and which are immersed in the water bath 'b' at 38°C. The gas flow is adjusted to approximately 10 ml./minute. Each gas is dispersed into fine bubbles by the

FIG. 1. Apparatus for equilibration of samples (see text).

sintered glass bulbs 'c' (A. Gallenkamp & Co. ref. no. 28 × 2; average pore size, 40-50 microns). Each then passes to the equilibration chambers 'd', which are the Pasteur pipettes containing the samples of blood. These are mounted in rubber bungs which occupy the open ends of 5 ml. glass syringes 'e'. In order to load the pipettes into the syringes, the latter are drawn up out of the water. With a finger on the upper end of the pipette, it is passed into the syringe and wedged into position as shown, making a gas-tight fit in the bung. The syringes are lowered into the water so that only the upper ends of the pipettes are above water level. Bubbles of gas rise through the blood and are prevented from reaching the top of the pipettes by the bent capillary tubes, 'f', which are placed in the pipettes and which have sealed lower ends. These capillary tubes cause the bubbles to burst before they reach the top of the pipette. Equilibration is continued for three minutes. The contents of each tube are then passed through the capillary electrode. The Pasteur pipettes and capillary tubes are discarded after use.

RESULTS

The accuracy of a pH measurement on a capillary sample of blood is affected by a number of factors. Important among these are the addition of heparin and dilution of the specimen because these are known to affect the acid-base state of the blood (Andersen, 1961). These factors were examined as follows in order to test the accuracy of the method of sampling.

Twelve capillary tubes were filled with heparin as described. One end was sealed and the tubes were stood upright. The volume of fluid and quantity of heparin present in each tube was calculated from the length of the heparin column which formed. The average amount of heparin present was 0.047 mg. (range 0.035 to 0.069 mg.). If exactly 0.1 ml. of blood were used to fill each tube, the dilution factor due to the heparin solution would be 4.7% (range 3.5 to 6.9%), and the average concentration of heparin 0.47 mg./ml. (range 0.35 to 0.69 mg./ml.). These figures do not allow for the amount of heparin adhering to the wall of the tube. The tubes were therefore weighed together before and after the addition of heparin and a mean figure was obtained for total heparin present in each tube. From this the actual heparin present and the true dilution factor were obtained by scaling up the figures above by 16%.

HEPARIN EFFECT Andersen found that when blood was added to dried heparin under anaerobic conditions to give a concentration of 1 mg./ml. there was a fall in pH of 0.003 units, a fall in base excess of 0.2 mEq./l., and an insignificant increase of PCO₂ of 0.1 mm. Hg (Andersen, 1961). Using a

similar technique but larger concentrations of heparin, we obtained similar results (Table I).

TABLE I

CHANGE OF BLOOD pH, PCO₂, AND STANDARD BICARBONATE AFTER ADDITION OF DRIED HEPARIN

Experiment	Heparin (mg./ml.)	pH Units	PCO ₂ (mm. Hg)	Standard Bicarbonate (mEq./l.)
1	2.5	-0.005	0	-0.2
2	2.5	-0.008	+0.5	-0.2
3	5.0	-0.015	-0.5	-0.5
4	5.0	-0.015	-1.0	-0.5
5	7.5	-0.020	-0.5	-0.7
6	7.5	-0.015	-1.0	-0.7

Varying amounts of dried heparin were added to blood and each sample was equilibrated with 2.67% CO₂ before the measurement of pH. The effect of heparin on pH is almost linear (Fig. 2), and less than 1 mg./ml. of blood has a negligible effect on pH. Heparin in the concentration used is unlikely therefore to produce an appreciable error.

FIG. 2. Effect of heparin on pH of blood.

DILUTION EFFECT When increasing amounts of normal saline are added to blood under anaerobic conditions, there is a fairly linear relationship between the percentage dilution and the fall of PCO₂ and standard bicarbonate, with a negligible change of pH. Table II shows the effect of a 10% dilution of blood with normal saline under anaerobic conditions. The error due to dilution with the method described will become appreciable if less

TABLE II

EFFECT OF DILUTION

Four Experiments	pH Units	PCO ₂ (mm. Hg)	Standard Bicarbonate (mEq./l.)
Initial values (mean + range)	6.998 (6.999-6.993)	94 (91-97)	15.2 (15-15.3)
10% dilution (mean + range)	7.001 (6.995-7.008)	79 (76-81)	14.4 (14.3-14.8)

than 0.1 ml. of blood is collected in the tubes and if care is not taken to remove all excess heparin from them before collection of the specimens.

TRANSFER TO CAPILLARY TUBES Appreciable amounts of CO_2 may be lost during collection when blood is transferred to the capillary tube. Table III shows the falls of pH when samples of blood equilibrated in Pasteur pipettes were transferred to capillary tubes. This error can be minimized as described by Andersen *et al.* (1960) by ensuring that the limb is warm so that the blood flows freely into the end of the capillary tube placed in contact with the skin puncture.

TABLE III

HEPARINIZED BLOOD EQUILIBRATED WITH 9.97% CO_2		
Experiments	pH	pH after Transfer to Capillary Tubes
1	7.068	7.084
		7.084
2	7.065	7.083
		7.084
3	7.069	7.081
		7.083

EFFECT OF STORAGE Ten capillary tubes were filled with heparinized blood and were allowed to stand on the bench for 10 minutes. The CO_2 tension was high (71 to 96 mm. Hg) in all but one sample. The change in PCO_2 and standard bicarbonate was not significant at the end of 10 minutes ($P > 0.1$). Two samples showed a fall of PCO_2 from 94 to 85 mm. Hg and 96 to 92 mm. Hg, but the others all agreed within 2 mm. The biggest difference in standard bicarbonate was 0.2 mEq./l.

Significant changes may occur when the blood is stored for longer periods and it is therefore recommended that the sample be examined within 10 minutes of collection.

EQUILIBRATION Samples of blood with pH between 6.94 and 7.31 units were equilibrated at different levels of CO_2 tension by the method described. Equilibration was complete in all cases within three minutes (Fig. 3).

OVERALL ERROR Twelve comparisons between samples collected simultaneously from an artery and the finger were made in heparinized subjects during cardiac surgery. The syringes for arterial samples were unheparinized and the capillary tubes were heparinized by the method described. In four, the limb was cold and bled with difficulty. In all but one of these there was good agreement between

FIG. 3. Equilibration of 10 samples of blood with carbon dioxide.

pH, PCO_2 , and standard bicarbonate and in this subject the PCO_2 was 34 mm. Hg (arterial, 22 mm. Hg) and the standard bicarbonates were identical. Taking the group as a whole, the pH, PCO_2 , and standard bicarbonate did not differ significantly ($p > 0.1$).

It is concluded that the various factors examined do not produce appreciable error providing the points of technique described are accurately adhered to.

DISCUSSION

The method of collecting samples in open-ended capillary tubes containing wet heparin has certain advantages. Clotting rarely occurs and mixing immediately after collection is unnecessary. We have demonstrated that the errors involved in this technique are acceptable for clinical purposes.

The method of equilibration described here also has certain advantages. The equilibration chambers have proved easy to use and are efficient. They are discarded after use so that the equipment does not require servicing between estimations. This is an advantage when frequent estimations are necessary. If large numbers of samples are being handled a series of equilibration chambers can be set up. We have found the method particularly valuable in the child when a series of estimations has been necessary.

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