The influence of extraneous factors on Coulter S measurement of the mean corpuscular volume

M. YOUNG AND A. C. K. LAWRENCE

From the Haematology Department, Northern General Hospital, Sheffield, England

SYNOPSIS Alteration in the temperature of the Isoton diluent in a Coulter model S counter over a range of possible laboratory working temperatures produced a change in the mean corpuscular volume using EDTA and dipotassium acid citrate dextrose blood and a commercial control, 4C. Contamination of the Isoton diluent with hypochlorite solution, used to clean the Coulter model S, may increase the mean corpuscular volume of EDTA blood without altering other measured parameters. Attention to both these points is required in assessing quality and calibrating the machine.

The Coulter counter model S (Coulter S) measures the mean corpuscular volume (MCV) in a direct fashion by comparing the mean voltage of pulses generated by cells passing through an aperture with the mean voltages generated in the same way by cells from a reference blood preparation. The variance of these measurements is very small compared with manual methods. Pinkerton, Spence, Ogilvie, Ronald, Marchant, and Ray (1970), Hattersley, Gerard, Caggiano, and Nash (1971), and Komarmy, and Barnes (1972) noted that the MCV may be spuriously high in the presence of high-titre cold agglutinins. This effect was avoided using a diluent warmed to 30°C. In the course of their report, the latter authors noted that recalibration of the Coulter S was required when using warmed diluent. The change of calibration occurring with varying diluent temperature has been investigated.

The weekly use of bleaching cleansing agents such as Chloros solution to remove protein from the aperture baths has been recommended by the manufacturers of the Coulter S. It is recommended that bleach solution be removed by repeated washing because of interference with cell and haemoglobin measurements. While the effect of small concentrations of cleansing agents is usually obvious, producing high and sometimes impossibly high, results in each parameter, the effect of small concentrations, in certain circumstances, on the MCV is less well known. An effect of small concentrations of blood on the MCV is illustrated.

Apparatus and Methods

The Coulter model S machines have been in routine use for about five years, and were used in accordance with the manufacturer's instructions. The diluent used was Isoton supplied by Coulter Electronics Ltd. Isoton was found to have a pH of 7.4.

The experiments were carried out in the second half of the working day when the machine was apparently functioning satisfactorily in routine use, and when results were being obtained using the 4C control material which were within 1 femtolitre of the assigned value of the mean corpuscular volume (MCV) and within 0.5 picogrammes of the assigned value of the mean cell haemoglobin (MCH) at the ambient temperature. All the measurements were made by one experienced worker.

Blood samples were taken into plastic containers. The anticoagulant used was either dipotassium EDTA with a final concentration of 4.2 mmol/l of blood or acid citrate dextrose (ACD) solution 1.0 ml to 3.5 ml of blood. The ACD solution consisted of disodium acid citrate (monohydric) 75 mmol/l and dextrose (anhydrous) 139 mmol/l. A commercial control preparation (4C Coulter Electronics Ltd) and a sample of red cells diluted in Isoton diluent issued for the Haematology Proficiency Assessments sponsored by the Department of Health and Social Security and the British Committee for Standardization in Haematology were also used.

The Chloros solution was a commercially prepared 10% wt/vol solution of sodium hypochlorite.

Effect of Mixing and Oxygenation on MCV

Samples of blood freshly taken into EDTA and an ACD solution and samples of blood stored for 21 days in ACD solution at 4°C were each taken in two 30 ml Universal containers. One container was full and the other contained approximately 7 ml of blood. The containers were mixed continuously for four hours at 21°C and tested periodically. There was no significant change in the MCV during the test period. It was concluded that mixing and oxygenation had no effect on the MCV as measured by the Coulter S.

Results**DILUENT TEMPERATURE**

Isoton diluent was brought to various temperatures in either glass flasks or in the plastic carton in which the diluent was supplied. Without altering the calibration of the instrument, the temperature of the red cell aperture bath was measured. Two blood samples, one taken in EDTA and one in ACD, were then measured, together with the control preparation 4C. This was followed by a second temperature measurement in the aperture bath. The temperature before and after each group of measurements differed by between 0 and 1.5°C.

Three groups of experiments were carried out on three separate days using different blood samples and control preparations and the results are presented in table I. The measurements of the MCV in experiments 1 and 2 were made in duplicate and mean results are presented with the mean aperture bath temperatures. The measured MCV of blood in EDTA and in ACD and of the commercial control material was higher when the diluent was cooled. Over a temperature range of 17.5 to 28.5°C, representing possible laboratory ambient temperatures, the difference of MCV was between 2 and 4 femtolitres. There was no trend of change in the MCH. The effect is similar in experiment 2 comparing an average room temperature of 22°C with a temperature of 32°C which could be used to avoid cold agglutination.

Experiment 3 shows that the change occurs in ACD blood with a temperature change of 4°C from 25 to 29°C and the MCV returns to the former value when the temperature is returned to 25°C. The same sample was measured 20 times at each temperature and the differences of the means were shown to be statistically significant ($P < 0.001$). There was a smaller rise in the mean MCH throughout the experiment which was statistically significant ($P < 0.01$) but was independent of temperature.

Experiment 4 shows that a similar change occurs

Temperature (°C)	EDTA Anticoagulant		ACD Anticoagulant		Control Preparation 4C	
	MCV (fl)	MCH (pg)	MCV (fl)	MCH (pg)	MCV (fl)	MCH (fg)
<i>Experiment 1</i>						
13	89	31.1	97	29.6	89	30.8
17.5	87	29.5	93	28.4	87	29.6
20	87	30.3	90	28.7	86	29.8
25	85	29.5	89	29.6	84	29.4
28.5	85	30.7	89	29.2		Not tested
35	84	28.7	86	28.4		Not tested
<i>Experiment 2</i>						
22	98	31.5	95	30.3	88	31.5
32	94	32.0	92	30.6	84	31.2
<i>Experiment 3</i>						
25(a)	—	—	80	25.9	—	—
29	—	—	78	26.3	—	—
25(b)	—	—	80	26.7	—	—
<i>Experiment 4</i>						
	<i>DHSS/BCSH Haematology Quality Assessment Scheme Blood Preparation</i>					
23	94	30.3	88	29.5	91	30.3
34	91	29.3	85	27.6	89	29.1

Table I Effect of temperature of diluent on the MCV of blood samples in EDTA and ACD and on the MCV of a control preparation

Probability of occurrence of difference by chance

Between MCV at 25(a) and 29 $t = 10.9$ $P = < 0.001$

Between MCV at 29 and 25(b) $t = 11.2$ $P = < 0.001$

Between MCH at 25(a) and 29 $t = 3.0$ $P = < 0.01$

Between MCH at 29 and 25(b) $t = 3.0$ $P = < 0.01$

Addition	White Count ($\times 10^9/l$)	Red Count ($\times 10^{12}/l$)	Haemoglobin (g/dl)	MCV (fl)	pH
Blood Sample I					
No bleach	3.3	3.15	10.2	89	
0.5% Chloros solution	4.0	3.4	—	92	
1% Chloros solution	4.0	3.11	10.4	99	
5% Chloros solution	—	3.12	14.1	130	
Blood Sample II					
No bleach		3.66	10.5	85	7.4
0.1% Chloros solution		3.70	10.5	94	7.45
0.5% Chloros solution		3.44	14.8	102	7.55

Table II Effect of addition of 0.1 ml volumes of bleach solutions to 10 ml volumes of blood diluted in Isoton diluent¹

¹See text for calculation of bleach concentration.

with a diluted suspension of cells used for a national quality assessment scheme. In this experiment ACD blood and the 4C preparation were included for comparison.

Isoton heated to 37°C for one hour and cooled to 0°C for one hour and then returned to room temperature had no effect on the result of the MCV, indicating that no significant permanent change in the composition of the diluent had taken place when the temperature had been altered.

Varying the temperature between 20 and 30°C had no effect on the precision of the MCV and MCH measurements.

EFFECT OF BLEACH

Dilutions of Chloros solution freshly received from the suppliers were prepared ranging from 0.5 ml of Chloros made up to 100 ml of water to 10 ml of Chloros made up to 100 ml of water. These dilutions are referred to as 0.5% Chloros and 10% Chloros respectively. The concentrations of intermediate dilutions were derived in the same way.

Of an EDTA blood sample, 44.7 μ l was diluted in 10 ml of Isoton diluent and then 0.1 ml of each Chloros dilution added. These suspensions were measured without delay using the capillary aspirator of the Coulter S and were compared with a similar blood dilution containing no bleach.

Table II shows that when blood diluted in 10 ml of Isoton diluent encountered small quantities of Chloros there was no change in the measured haemoglobin concentrate, red cell count, or white cell count compared with blood dilutions containing no bleach. Increasing amounts of Chloros solution produced significant changes in the MCV before there was noticeable change in the other parameters and before there was a significant change in the pH.

The MCV of blood dilutions made in a range of isosmotic phosphate buffer solutions (Dacie and Lewis, 1970) was unchanged over the range of pH

7.3 to 7.9 and was similar to the MCV found after dilution of the same blood in Isoton pH 7.4.

Discussion

The volume of red cells depends on the osmolarity of the diluent and the pH (Parpart, Lorenz, Parpart, Gregg, and Chase, 1947; Collier, 1968; Helleman, 1972a). The volume of the red cell in phosphate buffer mixtures is at a minimum at a pH of about 8 and if the pH falls below 7 the red cells swell significantly and immediately.

Isoton has been recommended as a diluent by Helleman (1972b) in reporting on a trial of erythrocyte counting organized by the International Committee for Standardization in Haematology. Isoton is a modified Eagle's solution and consists of 142 mmol of sodium chloride, 5.5 mmol of potassium chloride, calcium chloride, disodium hydrogen phosphate, 154 mmol of sodium azide and glucose, together with water to make a volume of 1 litre, followed by sufficient 3 mol/l hydrochloric acid to adjust the pH to 7.4 (Helleman, 1972b).

Brecher, Jakobek, Schneiderman, Williams, and Schmidt (1962) showed that Eagle's solution yielded values for the MCV using a Coulter counter model B which were similar to measurements made of red cells in AB plasma.

The possible causes of the fall in MCV measurements with the rise in diluent temperature include changes in the aperture, the flow pattern, and detector system, changes in the diluent, or changes in the red cells.

It seems unlikely that there is a physical change in the aperture, the flow pattern, or the detector system with such small variations in temperature.

A rise in the conductivity of the diluent can be expected with a rise in the temperature coefficient of Eagle's solution. Assuming that the conductivity of red cells is very low, the differential change in

conductivity between a given red cell and electrolyte induced by a rise of temperature would increase. This would produce the effect of an increase in mean corpuscular volume.

Contamination of the diluent in an aperture bath may alter the mean corpuscular volume. An example of this involving bleach was found during this investigation and is described. Bleach may significantly alter the MCV without significantly altering the pH of the diluent leading to high results for the MCV alone. This may be the source of errors in calibration, and this cause for minor changes in the MCV alone should be considered before recalibrating for this parameter. However, contamination does not explain the reproducible phenomenon of alteration in the measured MCV with diluent temperature.

It is possible that heating or cooling of the Isoton produces a chemical change in the diluent but prolonged heating and cooling of samples of Isoton did not alter the suitability of these samples to measure the mean corpuscular volume. The effect of temperature therefore is not due to permanent chemical alteration of the composition of Isoton by heating or cooling.

Red cells do not appear to change in size with mixing or oxygenation but may change in size with temperature. This is a likely but unproven possibility. The MCV measurements using the Coulter S fell when the diluent was warmed. This occurred using fresh blood in EDTA or ACD solution or using a commercial control preparation of human red cells, 4C. This phenomenon is of significance in assessing performance throughout the day using a working reference preparation or in duplicate and check testing of samples using the Coulter S. The change with temperature change is particularly important when calibrating the machine and has been largely ignored. The manufacturers of the control preparation, 4C, state on the sheet accompanying the material that the product should not be frozen and that it maintains the assay values after refrigeration, but no indication is given of the temperature at

which the MCV value assigned may be obtained using the Coulter S. The material used in the Department of Health and Social Security and British Committee for Standardization in Haematology quality assessment scheme is taken into an ACD solution. Such suspensions are susceptible to change of MCV with the temperature of the diluent in the Coulter S.

In accordance with the suggestion of Hattersley *et al* (1971) it is concluded that temperature control of the Coulter model S is required not only to avoid cold agglutination but to define and maintain the calibration of the mean corpuscular volume. It is suggested that attention be given to defining a single suitable temperature for use with all Coulter model S counters and the introduction of a modification to ensure the maintenance of this temperature within 2°C.

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