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The paper punched disc technique for lead in blood samples with abnormal haemoglobin values

G. F. CARTER

From the Regional Toxicology Laboratory, Dudley Road Hospital, Birmingham B18 7QH

ABSTRACT A series of 15 blood samples with haemoglobin levels ranging from 4·6–16·1 g/dl were spotted on to Whatman No. 4 filter paper. Blood samples with low haemoglobin concentrations spread over a greater area of the filter paper than did those with high haemoglobin concentrations. This was further investigated by studying the performance of laboratory-prepared samples, and any effect on the estimation of blood lead value. Blood lead values assayed by the punched disc method on blood samples with low haemoglobin values were unreliable unless the estimated value was adjusted with respect to the area over which the blood had spread.

The method of dropping blood on to filter paper and allowing this to spread and dry before punching out discs of uniform size has been recommended as an alternative to volumetric measurements of blood (Cernik and Sayers, 1971, 1973; Fox and Sayers, 1973; Cernik, 1973a, 1973b, 1974). Although the effect of environmental features such as humidity while spotting the blood, and temperature of drying the filter paper, have been closely examined (Cernik and Sayers, 1971), very little investigation seems to have been carried out on the effect of anaemia upon the size of spot produced.

Cernik (1973b) refers to an 'admittedly few' bloods with haemoglobin values between 9 and 11 g/dl giving no significant difference in blood lead values estimated by both the punched disc technique and cathode ray polarography.

During the four years that the punched disc method of blood sampling was used in this laboratory it was noted that blood samples of low haemoglobin concentration spread over a larger area than did blood samples with a normal haemoglobin concentration. This was investigated in two ways. First, 15 blood samples covering as wide a range of haemoglobin values as possible, all of which had been collected and routinely examined on the same day, were spotted on to filter paper and the area of spread measured. Second, various dilutions of a red cell concentrate with plasma were prepared. These dilutions were then examined for haemoglobin concentration, packed cell volume (PCV), red cell count (RBC) and lead concentration. Two spare filter paper discs were prepared for each sample so that the area of spread could be determined.

After the second series of samples had been prepared and examined it was decided to prepare a further series of dilutions of red cells in plasma, all of which would have the same lead content. These were examined in the same way as the previously prepared samples.

After using 100 μl of blood for spotting on to filter paper it was brought to the author's attention that this contravened recommendations made by Ainsworth *et al.* (1953) who stated that restricted volumes only, 20–60 μl, gave uniformity of spread.

In order to validate the use of 100 μl samples, two blood samples with haemoglobin values of 4·5 and 16·5 g/dl were examined for areas of spread, using both 50 μl and 100 μl sample sizes.

Materials and methods

The Haematology Department of Dudley Road Hospital supplied the first 15 samples which had been assayed for haemoglobin and PCV. For the preparation of the other two sets of samples 500 ml of blood was collected into EDTA (dipotassium salt) and partially separated into plasma and a cell concentrate by centrifugation. To the cell concentrate was added 200 μl of a solution containing 200 μg of lead (as lead chloride). This was then mixed for four hours using a magnetic stirrer. It was estimated that this procedure would raise the lead content of the cell concentrate to approximately 100 μg/dl. This was then combined with the separated plasma as shown in Table 1.

For the second series of laboratory-prepared

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Table 1 *Details of preparation of laboratory samples*

Composition	*Sample code*										
	B0 A0	*B1 A1*	*B2 A2*	*B3 A3*	*B4 A4*	*B5 A5*	*B6 A6*	*B7 A7*	*B8 A8*	*B9 A9*	*B10 A10*
Cell concentrate (ml)	0	1	2	3	4	5	6	7	8	9	10
*Plasma (ml)	10	9	8	7	6	5	4	3	2	1	0

*For second set of dilutions spiked plasma was used (see text).

samples, sufficient lead chloride was added to the separated plasma to raise the lead level to equal that of the cell concentrate. This was achieved by dropwise addition of lead chloride solution to the plasma until the peak height of assay was equal to that of the red cell concentrate. Dilutions of the cell concentrate were then prepared as for the first set.

In order to verify that there were no significant differences in matrix effects between the cell concentrate and its plasma, known quantities of lead chloride solution were added in increasing concentration to both the cell concentrate and to its plasma. The calibration curves obtained were identical and therefore it was assumed that the matrix effects were not significantly different.

Blood lead levels were measured by the Delves cup technique (Delves, 1970) with three modifications: hydrogen peroxide was not used for preliminary oxidation of the sample, because this was not necessary when background correction was used; measurements were made at a wavelength of 217·0 nm; and the samples were dried at 150°C for 30 seconds on a Gallenkamp hotplate.

For the assay of lead by the punched disc technique, blood samples were spotted as follows: 100 μl of blood was dropped from an Oxford pipette on to a Whatman No. 4 filter paper (7 cm diameter) from a height of 1 cm. The papers were then placed into plastic disposable Petri dishes and allowed to dry overnight. A 6 mm disc was punched from the centre of each blood spot and inserted into a nickel cup for analysis.

For the experiment to validate the usage of 100 μl of blood for spotting on to filter paper, the above procedure was followed using both 50 μl and 100 μl of samples with haemoglobin values of 4·5 and 16·5 g/dl. This was performed in quadruplicate.

When measuring blood lead, a set of standards was estimated by both the volumetric and the punched disc filter paper techniques to check that linearity applied for all relevant levels of lead. These standards covered the range 20–120 μg/dl.

All blood lead measurements were carried out in duplicate. Actual lead concentrations of the prepared samples were not estimated; instead, for each set the cell concentrates were designated as having a value of 100 and the concentrations in the other samples were calculated from this.

The area of spread was measured by inserting a sheet of carbon copy paper between the filter paper on to which the blood had been spotted, and a piece

Table 2 *Results from random samples*

Haemoglobin (g/dl)	*PCV (%)*	**Area (mm²)*
16·1	44	400
15·7	47	354
15·6	43	470
15·4	44	373
13·4	36	516
13·2	38	547
12·4	37	593
12·2	38	612
11·9	37	621
11·6	33	768
10·7	30	828
9·8	31	826
8·1	25	860
6·3	21	916
4·6	14	963

*Area = average of two results.

Table 3 *Results from first series of laboratory-prepared samples*

Sample no.	*Hb (g/dl)*	*PCV (%)*	*Area of spread (mm²)*	**Lead by volumetric assay*	**Lead by punched disc technique*	*Expected result*
A10	18·4	54·4	266	100	100	100
A9	16·1	48·7	336	87	81	90
A8	14·9	45·5	392	77	40	80
A7	12·7	38·4	578	68	32	70
A6	11·0	33 3	748	57	26	60
A5	9·2	28·3	809	50	18	50
A4	7·2	21·8	849	39	13	40
A3	5·3	16·7	928	31	8	30
A2	3·8	11·8	920	21	8	20
A1	1·9	6·1	1021	13	4	10
A0	0·1	1·3	1030	3	2	0

*Arbitrary units.

Table 4 *Results from second series of laboratory-prepared samples*

Sample no.	*Hb (g/dl)*	*PCV (%)*	*Area ($\bar{x}$ of 3)*	**Lead by volumetric assay (A)*	**Lead by punched disc technique (B)*	*A-B*	*Expected result*
B10	18·9	53·8	365	100	100	0	100
B9	17·1	49·3	430	99	82	17	100
B8	15·0	43·4	481	98	72	26	100
B7	13·0	37 5	654	97	69	28	100
B6	11·5	33·0	730	97	56	41	100
B5	9·5	28 1	761	98	48	50	100
B4	7·9	22·5	853	96	40	56	100
B3	5 9	17·4	913	99	39	60	100
B2	4·0	11·6	982	98	36	62	100
B1	2·2	6·4	1017	99	35	64	100
B0	0·4	1·6	1064	98	33	65	100

*Arbitrary units.

Table 5 *Preparation of blood spots using two volumes of bloods with different haemoglobin concentrations*

Volume of blood sample (µl)	*Area of spot (mm²) for blood with Hb values of:*	
	4·5 g/dl	*16·5 g/dl*
50	504, 493, 475, 501 — mean = 493; *SD = ± 13·0; †CV = 2·6	176, 167, 181, 212 — mean = 184; SD = ± 19·5; CV = 10·6
100	991, 986, 987, 1009 — mean = 993; SD = ± 10·7; CV = 1·1	426, 476, 414, 418 — mean = 434; SD = ± 28·8; CV = 6·6

*Standard deviation.
†Coefficient of variation.

of millimetre-squared graph paper. The perimeter of the blood-stained portion was traced and the area enclosed was estimated.

INSTRUMENTS

All blood lead measurements were made using an Instrumentation Laboratory Model 353 Atomic Absorption Spectrophotometer fitted with an IL Microsampler.

The haematological estimations were carried out on a Coulter Model S.

Nickel cups were obtained from Electronic Developments Ltd, Two Stacks, Priors Wood, Guildford.

Results

Table 2 shows the haemoglobin concentrations, PCVs and areas of spread of the blood samples supplied by the Haematology Department. Tables 3 and 4 show results of blood lead estimations in which unspiked and spiked plasma respectively were used for dilution. The results of the experiment to validate the use of 100 µl blood spots are shown in Table 5.

Figure 1 shows the area of spread plotted against haemoglobin and PCV values, and Figure 2 shows the discrepancy between the results of blood lead estimations using a volumetric technique and those obtained by the punched disc technique, according to the concentration of haemoglobin in the blood sample. Examples of the size of spot produced are shown in Figures 3–6.

Discussion

The method of spotting blood on to filter paper has been used as a convenient means of surveying industrial and other populations. In making such preparations it is clearly important to make the collection under the most stringently hygienic conditions. These conditions have been exhaustively discussed by many authors since Cernik and Sayers stated them clearly in 1971.

The CEMA's Note of Guidance (1972) gives information concerning the interpretation of lead levels, haemoglobin levels and a correction nomogram for the lead/PCV relationship. During population studies in this laboratory it was noted that, for a constant dropping height of blood on to filter paper, different areas of spread of blood spot were obtained. Knowing that haemoglobin content may vary, particularly in women and children of various ages,

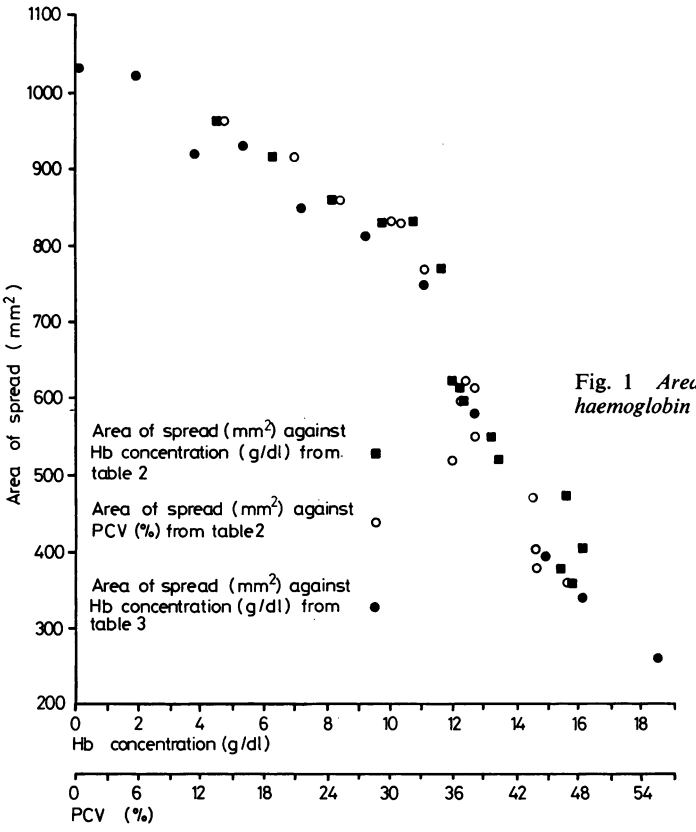

Fig. 1 *Areas of spread of blood plotted against haemoglobin concentration and packed cell volume.*

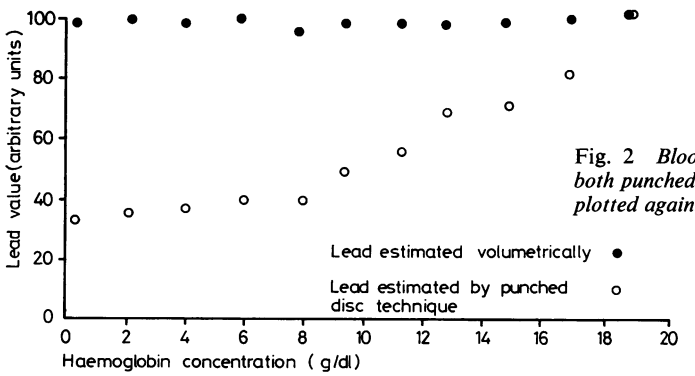

Fig. 2 *Blood lead values (arbitrary units) obtained by both punched disc technique and volumetric measurement, plotted against haemoglobin concentration.*

we thought it advisable to investigate this further.

The use of 100 μl samples is justified by the results obtained in comparing areas of spots produced by using both 50 and 100 μl quantities of blood.

The results of this investigation show (Table 2) that, in a series of random samples from the Haematology Department at this hospital, the area of spot varied both with haemoglobin concentration and PCV.

Unfortunately, after having estimated the area of spread of these samples the filter papers were considered to have been contaminated to such an extent

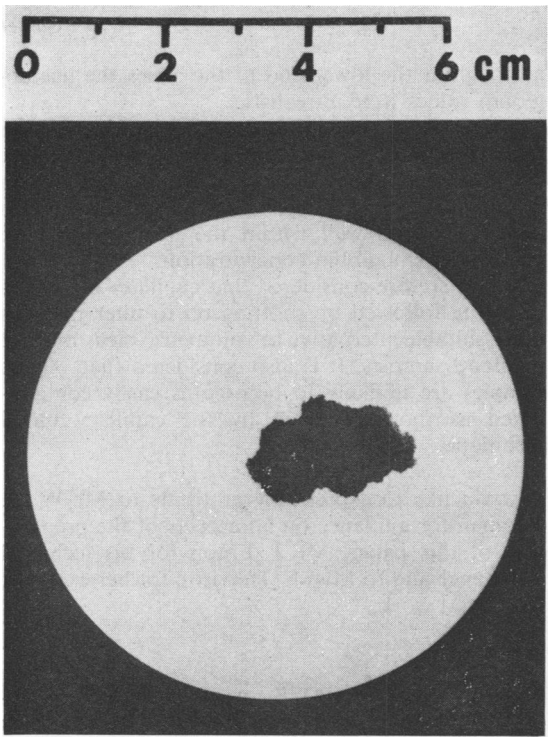

Fig. 3 *50 μl of blood, haemoglobin concentration 16·5 g/dl, spotted on to Whatman No. 4 filter paper.*

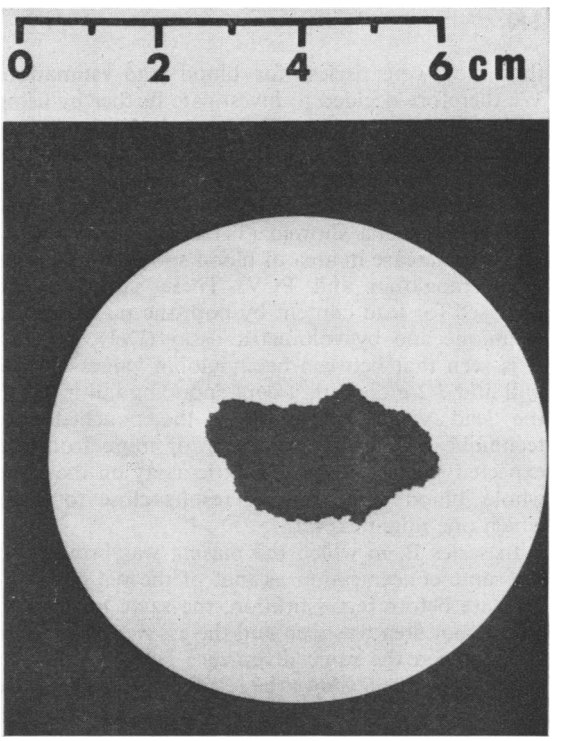

Fig. 4 *100 μl of blood, haemoglobin concentration 16·5 g/dl, spotted on to Whatman No. 4 filter paper.*

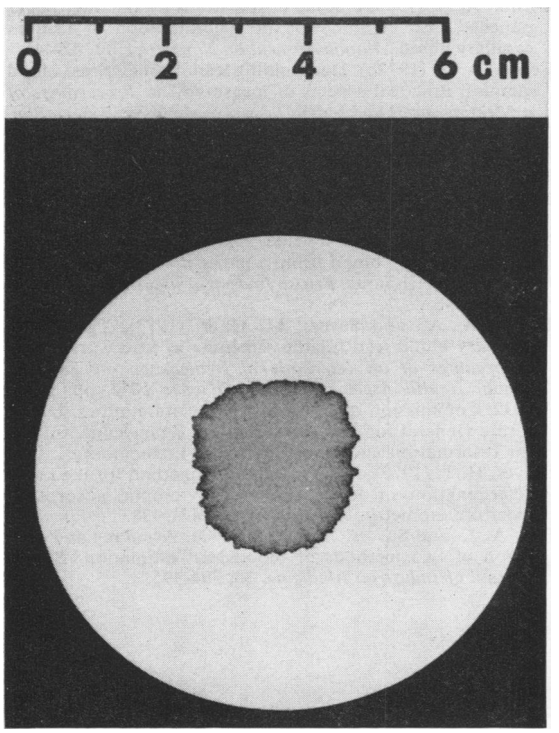

Fig. 5 *50 μl of blood, haemoglobin concentration 4·5 g/dl, spotted on to Whatman No. 4 filter paper.*

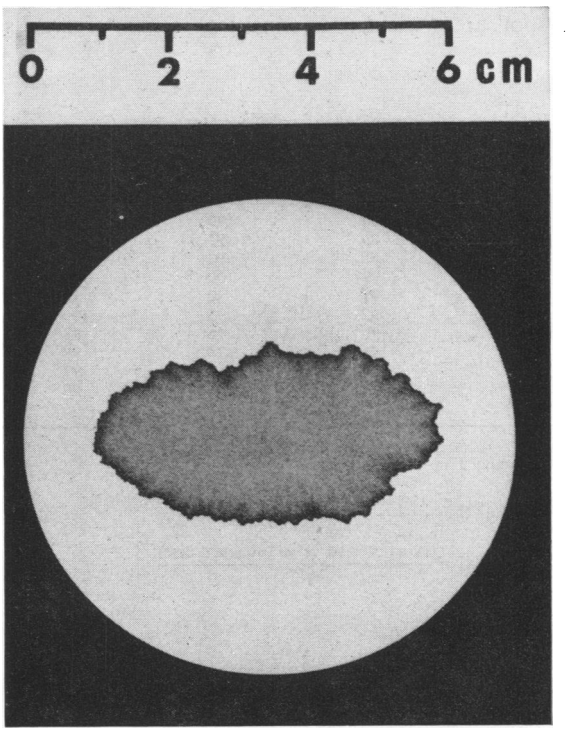

Fig. 6 *100 μl of blood, haemoglobin concentration 4·5 g/dl, spotted on to Whatman No. 4 filter paper.*

that they were useless for blood lead estimation. We therefore decided to investigate further by using laboratory-prepared samples in which all the factors under consideration could be more closely controlled.

The artificial series A in which lead was added to the red cell concentrate before volumetric reconstitution with plasma showed (Table 3) a similar progressive increase in area of blood spot with decrease in haemoglobin and PCV. These samples were analysed for lead content by both the punched disc technique and by volumetric assay (Delves, 1970). It is seen that between haemoglobin values of 14·9 g/dl and 7·2 g/dl, with a corresponding fall in PCV, the lead value estimated by the punched disc technique may deviate by 50% or more from the expected values. The volumetric assay on the same whole blood samples gave results close to those which one might expect.

In series B, in which the plasma was brought to the same concentration as that of the red cell concentrate before reconstitution, the same increase in blood spot area was seen and the assayed lead levels also showed the same divergence between the two methods. Table 6 shows the results of correcting the blood lead levels from the punched disc experiments for haemoglobin ratios, for PCV ratios and for relative areas taking the prime sample B10 as reference. Only the corrections for relative blood spot areas yielded a consistent series of results, although at the lower end of the series the haemoglobin values were unrealistic.

Table 6 *Comparison of results after correction*

Sample no.	*Column I (PCV)*	*Column II (Hb)*	*Column III (area)*
B10	100	100	100
B9	89	91	97
B8	89	91	95
B7	99	100	124
B6	91	92	112
B5	92	95	100
B4	96	96	93
B3	121	125	98
B2	167	170	97
B1	294	301	98
B0	1110	1560	96

$$\text{Column I} = \frac{53{\cdot}8 \times \text{lead value (punched disc)}}{\text{PCV (sample)}}$$

$$\text{Column II} = \frac{18{\cdot}9 \times \text{lead value (punched disc)}}{\text{Hb (sample)}}$$

$$\text{Column III} = \frac{\text{Area} \times \text{lead value (punched disc)}}{365}$$

Using the punched disc technique, unless the result obtained is corrected with reference to the spread of the standard blood used, the result may deviate considerably from the true value. This deviation starts well within the normal reference range of haemoglobin concentration.

It is therefore considered that capillary or venous sampling followed by spotting on to filter paper is not a suitable alternative to volumetric measurement of blood samples. It is also considered that venous samples are unlikely to become as easily contaminated as those obtained by the capillary blood technique.

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