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Determination of lead in capillary blood using a paper punched disc atomic absorption technique

Application to the supervision of lead workers

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Cernik, A. A., and Sayers, M. H. P. (1971). *Brit. J. industr. Med.*, 28, 392-398. Determination of lead in capillary blood using a paper punched disc atomic absorption technique. Application to the supervision of lead workers. The presence of lead in blood is the most incontrovertible evidence of absorption but hitherto the need for venepuncture has limited its determination in the supervision of industrial workers. Micro-methods using atomic absorption spectrophotometry (AAS) have, however, made possible the development of a sufficiently reliable test using a drop of blood obtainable by ear prick for use in the field for screening purposes.

A micro-sampling method by AAS is compared with a routine polarographic procedure (POL) using venous blood (corr. coeff. = 0·990). The pipetting of microlitres of blood can be eliminated by spotting the blood onto filter paper, allowing it to dry in air, and then using a punched-out standard disc of dried blood for analysis instead. Correlation of this method (PD) with the micro-sampling technique (AAS) is good (r = 0·981).

The PD method using capillary blood also correlates acceptably with the micromethod using venous blood (r = 0·913). A pilot field study using capillary blood estimated by the PD technique showed that with this method blood can be collected by ear prick in factories for monitoring workers in the lead industry, thus eliminating the need for routine venepuncture.

Many tests are available for screening workers exposed to lead and an extensive literature exists on their relative merits. The blood lead is generally considered the most incontrovertible evidence of lead absorption but the technical difficulties of analysis coupled with the need for regular venepuncture have precluded its general adoption.

Many of the problems of blood lead analysis are inherent in all the commonly used techniques and conventional atomic absorption spectrophotometry is no exception. The main disadvantages of the latter method for blood lead determination were its relatively poor sensitivity for lead and the inefficiency of the standard nebulizer-burner systems for producing atomic vapours. Hence there was a need for large sample volumes requiring venepuncture (Selander and Cramér, 1968; Cernik, 1970). The recent modification of the system for producing atomic vapours by the use of an atomic reservoir and tantalum boat has, however, greatly increased the sensitivity of atomic absorption spectrophotometry, reducing sample volumes to microlitres (White, 1968; Kahn, Peterson, and Schallis, 1968; West and Williams, 1969; Kahn and Sebestyen, 1970).

Delves (1970) determined lead in 10-μl blood samples over a range of 19 to 245 μg/100 ml and in 20-μl samples up to about 120 μg Pb/100 ml blood. He obtained a limit of detection of $1{\cdot}19 \times 10^{-10}$ g, and a sensitivity of $1{\cdot}10^{-10}$ g for 1% absorption. The method proved both rapid and accurate. He

adapted the methods of White (1968) and Kahn *et al.* (1968) by using a nickel microcrucible for the sample preparation and a nickel absorption tube in the flame to concentrate the atoms.

We have simplified Delves's technique still further with a view perhaps to automation. The possibility of overcoming the need to use venous samples by adapting the method to capillary blood has been investigated. Estimation of blood lead might prove more acceptable to industry in the screening of workers if venepuncture could be avoided.

The difficulties of collecting uncontaminated specimens of capillary blood by precise volumetric measurement in the factory require no elaboration and prompted the study of alternative techniques. The work of Guthrie (1961) with phenylalanine initiated the use of filter paper to eliminate volumetric measurements and to allow the use of capillary samples. Subsequent investigations by Hill and Palmer (1969) showed that results from discs of blood, punched from filter paper, for substances other than lead, such as tyrosine, glucose, and urea nitrogen, compared in precision with those obtained after careful pipetting. Elution of the sample was necessary for these analyses. In the work described here dried punched discs were analysed for lead directly without prior treatment.

The purpose of this paper is threefold:

(*a*) to compare the results of the method described by Delves (1970) using microlitre samples of venous blood with those given by a standard polarographic procedure for blood lead;

(*b*) to evaluate the use of the filter paper (punched disc) technique as a method of estimating the blood lead using venous samples;

(*c*) to evaluate the filter paper technique as a method of collection of capillary blood for estimating the blood lead with a view to its use in the supervision of lead workers.

Material and methods

Abbreviations and units

The three methods of analysis referred to in this paper are designated as follows:

AAS, the micro-method of Delves (1970) using 10 or 20 μl samples as indicated in the text

POL, a standard polarographic procedure as described by Cernik (1967)

PD, the new punched disc technique using venous or capillary blood spotted onto Whatman No. 4 filter paper as described in the text.

The units of measurement in the regression equations are given in μg Pb/100 ml blood.

Apparatus

A Perkin Elmer '303' atomic absorption spectrophotometer was used with the nickel crucibles and sampling unit similar to that described by Delves (1970), whose technique of analysis was followed in all essential details. The nickel absorption tubes were, however, replaced by more durable tubes made of aluminium oxide (Al_2O_3), 100 mm long by 18 mm internal diameter. The wider bore of the alumina tube extends the limit for 20 μl to about 140 μgPb/100 ml, but there is a resulting slight decrease in sensitivity. Polarographic determinations were carried out with a cathode ray polarograph using the method described by Cernik (1967).

Collection of specimens

Venous and capillary blood samples were used as indicated in the text. Venous samples were collected with 10-ml plastic syringes. Plastic ampoules containing 10 to 20 mg di-potassium ethylenediamine tetra-acetic acid as anticoagulant were used. Capillary blood was spotted onto Whatman's No. 4 filter paper (9 cm diameter) and dried in air. When this latter method was used for the collection of specimens from workers in factories, scrupulous attention to detail was necessary to prevent contamination with lead. The ear was thoroughly cleaned with lead-free soap and sterile swabs followed by the gentle use of nail and tooth brushes; the skin surface was then 'sterilized' with 0·05% Hibitane in alcohol and dried. The lower margin of the lobe of the ear was punctured with a stilette and the blood was allowed to flow freely and the ear was wiped again before blood was collected in a heparinized Harshaw tube. The blood was then expelled by gravity or by blowing[1] gently onto Whatman No. 4 filter paper in two or more locations.

To prevent contamination, the filter papers were enclosed in 6-in petri dishes and not exposed more than necessary during the spotting of the blood. Eight duplicate samples could be spotted easily on one piece of filter paper.

Analysis by punched disc

It was first necessary to obtain a disc of filter paper of an area which, when spotted with blood, would hold approximately 10 μl, a volume which had been shown by Delves (1970) to give a satisfactory working range for blood lead. This was determined in the first instance empirically with blood containing lead using a series of punches until one was found such that repeated determinations on the discs gave results close to those obtained with 10-μl samples measured volumetrically and estimated by the Delves (1970) technique.

The optimum punch size was then calculated mathematically on the basis of the experimental data and shown to be 8·9 mm diameter. This gave acceptable results over a range of 20 to 250 μg lead per 100 ml (Fig. 1). The punch used for cutting the discs from filter paper was an ordinary paper punch, modified to cut discs of the required size.[2]

In further confirmation that the size was correct, a 9·0-cm circle of Whatman No. 4 qualitative paper was weighed at ambient temperature (A). A 500-μl (0·5-ml) volume of blood was accurately pipetted onto the paper in multiple spots which merged and then were allowed to dry. The paper plus dried blood was then weighed (B).

[1] A rubber teat with a hole in the bulb as used with vials of vaccine lymph is suitable.

[2] Model 216 manufactured by Messrs Maun Industries Ltd, Moor Lane, Mansfield, Nottingham.

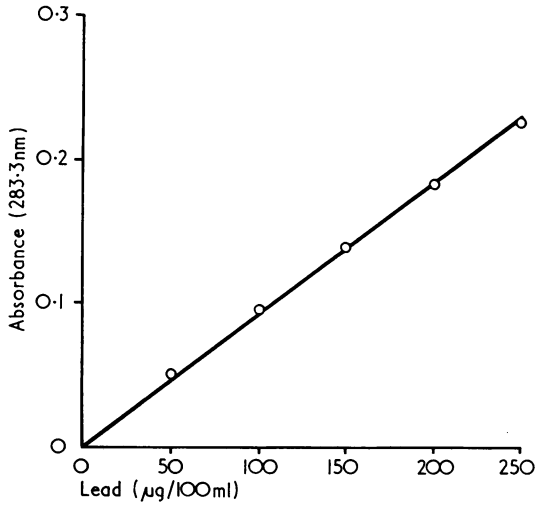

FIG. 1. Calibration curve for added lead in blood using the punched disc method. The curve goes through zero as the original lead content of the blood has been deducted.

The difference found (B-A) was the weight of dried blood from 500 µl (95·9 mg). Using the same blood sample and technique, a fresh blood-soaked circle of paper was dried, and fifty 8·9-mm discs were punched from it. The discs were weighed (C). Fifty blank discs were punched from a fresh piece of filter paper and the weight of the paper discs was determined (D). The difference found (C-D) was the weight of the dried blood from fifty 8·9 mm discs (96·0 mg). This difference is not significant and it can, therefore, be assumed that an 8·9 mm disc holds 10 µl of blood.

On Whatman No. 4 qualitative paper under the conditions described, the spread of blood was independent of the size of the drop applied. Experiments showed that discs cut at any position within a large spot gave the same result within the experimental error of the method. The effects of humidity and desiccation on the spread of blood through the filter paper were also studied. Whatman No. 4 filter paper was placed in an incubator maintained at 100% humidity and 37°C for four days. The paper was removed, blood spotted onto it, and allowed to dry in air under normal conditions; 8·9 mm discs were punched out and the lead was determined. Some paper from the same batch was placed over silica gel in a vacuum desiccator which was then evacuated. Four days later, the same blood sample used in the humidity experiments was spotted, 8·9-mm discs were cut, and the lead was determined. Table 1 shows the results of six replicate determinations of lead concentrations under these varying conditions of humidity. There was no significant difference between the mean values (*t* test).

The possibility of batch variation in the manufacture of the filter paper was also considered. Results using three different batches are given in Table 2 and showed no significant difference (*t* test).

TABLE 1

EFFECTS OF HUMIDITY ON THE UPTAKE OF BLOOD ONTO WHATMAN NO. 4 FILTER PAPER

Atmospheric conditions	*Mean concentration of lead (µg/100 ml)*	
	Sample 1	*Sample 2*
Dry	46	82
Humid	51	86
Laboratory ambient ..	49	86

No. of replicates in each case = 6.

Provided the punch is cleaned between each sample, by punching a fresh piece of filter paper several times, the blank is low enough to be ignored.

The punched out discs were placed in the nickel crucible without prior treatment other than drying in air and were introduced into the air-acetylene flame of the atomic absorption spectrophotometer below the opening in the aluminium oxide tube. The height of the absorption signal given by the lead atoms, which appears immediately after the non-specific absorption signal due to combustion products of the paper and blood, was recorded (Fig. 2).

Interpretation of peak height

When using venous blood, the ease with which the analyses can be done simplifies repetition in the event of failing to obtain an acceptable duplication of the recorded signal which occasionally occurs. When the blood lead is less than, say, 80 µg/100 ml, our practice is to accept a difference of up to 2% absorption in peak height. At higher blood lead concentrations we accept a difference in peak height of up to 3%. In both cases the mean is taken of the duplicates. Where, however, the difference is greater than this, the analysis must be repeated.

This failure to record acceptable duplication of the lead absorption signal tends to occur more commonly when using capillary than venous blood samples since there are inherent difficulties in their collection in the factory which have yet to be overcome. Such failures, however, are the exception and their occurrence would not appear to vitiate the use of capillary blood for a monitoring test. Moreover, our experience shows that the

TABLE 2

EFFECT OF DIFFERENT BATCHES ON THE UPTAKE OF BLOOD USING WHATMAN NO. 4 FILTER PAPER

Batch no.	*Mean concentration of lead (µg/100 ml)*
0073/02	98
8158/04	92
9086/06	98

No. of replicates in each case = 12.

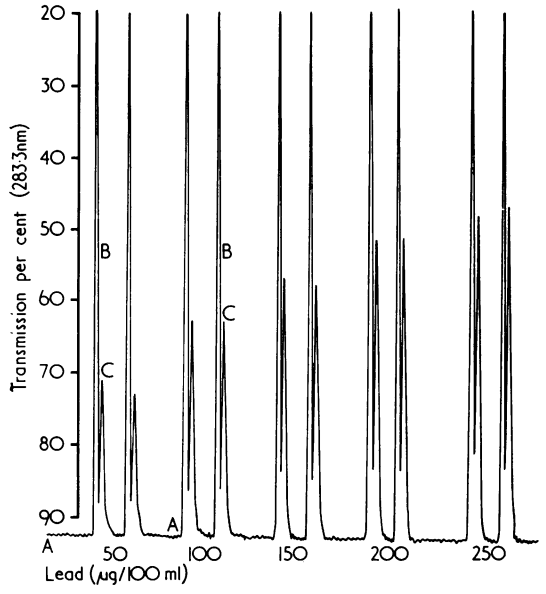

FIG. 2. Absorption signals at 283·3 nm obtained from duplicate 8·9 mm diameter punched discs containing 50 to 250 μg added lead per 100 ml pooled blood: (A) crucible introduced below tube in flame; (B) non-specific absorption signal from combustion products of paper and blood; (C) lead atomic absorption signal.

lower peak usually corresponds with the venous findings, suggesting that contamination with lead has occurred during collection of the capillary sample or its analysis. This, of course, is a well-recognized problem in all blood lead determinations and may require repetition of the analysis on a fresh sample.

Results

Comparison of micromethod and polarography

In the preliminary work we used 20 μl of blood and found good correlation between the results when compared with those given by a standard polarographic procedure. There was no significant difference between results given by the micromethod using either 10 or 20 μl samples of blood. These conclusions are based on an analysis of 51 venous samples with lead concentrations ranging from 17 to 114 μg/100 ml. The relationships derived by regression are expressed by the following two equations:

1. (20 μl) AAS = 0·903 × POL + 1·052; r = 0·975
2. (20 μl) AAS = 0·951 × (10 μl) AAS + 3·138; r = 0·990,

where r is the correlation coefficient.

TABLE 3

COMPARISON OF CATHODE RAY POLAROGRAPHY (POL) WITH THE MICROMETHOD (AAS)

Sample	*POL* (μg lead/100 ml blood)	*AAS* (μg lead/100 ml blood)
1	64	50
2	71	61
3	67	66
4	87	80
5	89	82
6	81	88
7	99	88
8	85	92
9	104	96
10	115	106
11	109	114
12	211	193
13	223	213
14	286	288

Each value is the mean of duplicate determinations.

POL = polarographic method (Cernik, 1967).
AAS = micromethod by atomic absorption spectrophotometry (Delves, 1970) using 10 μl samples.
Correlation (r) = 0·990.
Regression: AAS = 0·989 × POL − 3·945.
POL = 0·998 × AAS + 5·575.

Ten-microlitre samples were used in subsequent work in order to extend the upper concentration range of the analysis. The results for a series of venous samples with blood concentrations ranging from 50 to 288 μg/100 ml are given in Table 3.

Precision The results of 12 replicate determinations on samples of blood with added lead analysed by the micromethod (using 10 μl) at three different concentrations are given in Table 4.

Punched disc technique

Results using punched discs, each estimated to hold 10 μl of blood, have been compared with those using 10-μl samples measured volumetrically. Table 5

TABLE 4

PRECISION OF REPLICATE ANALYSES BY MICROMETHOD (AAS) USING 10-μL SAMPLES AT THREE DIFFERENT CONCENTRATIONS OF BLOOD LEAD

Mean (μg lead/100 ml)	*Standard deviation*
42	3·47
75	3·09
181	5·07

No. of replicates at each concentration = 12.

TABLE 5

COMPARISON OF THE MICROMETHOD (AAS) WITH THE PUNCHED DISC TECHNIQUE (PD) USING VENOUS BLOOD

Sample	*AAS* (*μg lead/100 ml blood*)	*PD* (*μg lead/100 ml blood*)
1	18	15
2	18	18
3	20	20
4	21	22
5	18	22
6	26	24
7	27	27
8	34	29
9	27	32
10	44	44
11	50	44
12	49	45
13	44	49
14	50	51
15	59	56
16	50	62
17	66	62
18	72	62
19	56	63
20	66	64
21	67	64
22	68	64
23	72	64
24	69	66
25	75	66
26	72	70
27	68	72
28	64	73
29	72	74
30	61	76
31	66	76
32	66	76
33	76	76
34	80	76
35	82	76
36	86	78
37	76	80
38	79	80
39	82	80
40	90	80
41	77	82
42	82	82
43	80	84
44	76	86
45	84	92
46	92	94
47	108	102
48	106	107
49	213	196
50	193	214

Each result is the mean of duplicate determinations.
AAS = micromethod using 10-μl sample by atomic absorption spectrophotometry (Delves, 1970).
PD = punched disc technique (for details see text).
Correlation (r) = 0·981.
Regression AAS = 0·975 × PD + 1·34.
PD = 0·988 × AAS + 1·20.

gives the comparative findings by the two methods in 50 samples of venous blood. Good correlation is observed over the approximate concentration range of 15 to 110 μg lead/100 ml blood with two readings at the 200 μg concentration.

Precision The results of 12 replicate determinations on samples of blood with added lead analysed by the punched disc technique at three different concentrations are given in Table 6.

TABLE 6

PRECISION OF REPLICATE ANALYSES BY PUNCHED DISC TECHNIQUE AT THREE DIFFERENT CONCENTRATIONS OF BLOOD LEAD

Mean (*μg lead/100 ml*)	*Standard deviation*
50	2·81
88	5·33
147	3·72

No. of replicates at each concentration = 12.

Use of filter paper for collection of capillary blood in the factory

Comparisons have been made on several occasions of results obtained using capillary blood collected on filter paper in the factory with those obtained at the same time using venous blood. The relationship derived by linear least squares regression from a typical set of results is given by the equation:

$$AAS = 0{\cdot}899 \times PD + 10{\cdot}35;\ r = 0{\cdot}913$$

This is the result of the examination of 100 lead workers in five factories using venous and capillary samples in each case. Unacceptable duplication (see page 395) was observed in 15 workers and these are excluded from the analysis.

This regression line with approximate 95% tolerance limits is given in Figure 3. It shows a small negative bias (about 10 μg/100 ml) by the disc method which is not fully understood. It may be due to a physiological effect of capillary blood or to dilution of the sample with tissue fluid or serum. It is also possible that it is due to a fractional deficiency in the area of the punched disc, although this bias was not as marked when using venous blood (see Table 5). If this is confirmed it should be readily corrected. The width of the tolerance limits is, of course, a reflection of the variations involved in both methods.

Discussion

The micromethod for the determination of blood lead has proved reliable and is simplified still further

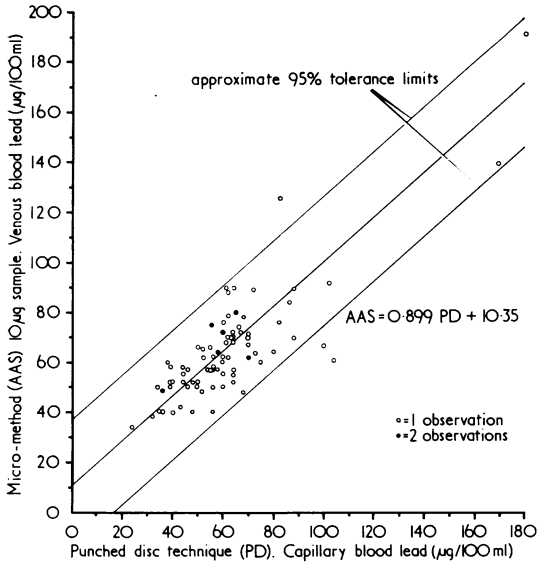

FIG. 3. A comparison of results obtained by the punched disc technique using capillary blood with those done by the micro-method using venous blood. The regression line is shown and the approximate 95% tolerance limits.

by spotting the sample onto filter paper and conducting the analyses directly on the punched out disc. This method eliminates errors of pipetting and renders the preliminary treatment of the sample unnecessary. Although the technique should lend itself to automation, the speed with which determinations can be done manually (say 30 samples per hour in duplicate) questions the possible advantage.

We have shown that it is possible, with scrupulous attention to detail, to collect samples of capillary blood in the factory which give results for blood lead comparable with those obtained from venous blood. The present deficiencies of the method are realized and it is evident that conditions in the factory affect the ease of collection of capillary samples which may be readily contaminated. This may result in unacceptable duplication in analysis. It is hoped that, by improving the technique, the number of such cases will be substantially reduced. Meanwhile the action to be taken when anomalies occur will depend on the purpose of the examination. If the capillary blood lead is to be used as an index of environment then it would seem reasonable to exclude such observations from the series and determine the group blood lead of the remainder. If, however, the history or results of clinical examination of the worker give rise to any doubt as to his fitness, then the only course is to repeat the examination using a venous sample, when other parameters can also be determined.

To illustrate the way the capillary blood lead could be used, we present in Table 7 a brief summary of the findings in three factories. Factory A is engaged in a new process of drawing wire, where the lead hazard is considered to be low. Factory B is manufacturing lead accumulators under fair conditions, and factory C is smelting lead, a process which is usually associated with a high risk. The group average of the capillary blood lead is shown in each case with that of the venous blood lead for comparison. It is seen that the figures reflect the conditions under which the men have been working. Three men with high blood leads in factory C were picked out for further investigation.

TABLE 7

CAPILLARY BLOOD LEAD AS AN INDEX OF ENVIRONMENT
(Venous blood lead results included for comparison)

Factory	*Lead hazard*	*No. of men (acceptable results/total)*	*Blood lead (µg/100 ml) (group average)*	
			Capillary (PD)	*Venous (AAS)*
A	Low	8 (8)	40	43
B	Medium	28 (33)	55	62
C	High	17 (20)	87[1]	91

[1]Three men with high blood leads (> 100 µg/100 ml) were picked out for further study.
AAS = micromethod by atomic absorption spectrophotometry (Delves, 1970).
PD = punched disc technique (for details see text).

These results are considered sufficiently encouraging to warrant further study of the capillary blood lead in the supervision of lead workers and their environment. It seems reasonable also to suggest that this method of collecting blood should prove heplful in paediatric practice by enabling the sample to be obtained with ease by heel prick where danger of contamination with lead should not exist.

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