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Results are presented for an interlaboratory proficiency study of blood lead determinations. Samples were pooled from individuals occupationally exposed to lead. Performance of individual laboratories is compared over a two year period. With increased emphasis in recent years on proficiency studies of this type, agreement between laboratories has been somewhat improved, but blood remains a difficult matrix in which to measure lead concentration.

Interlaboratory comparison of blood lead determinations

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Introduction

Of the several indices used for monitoring environmental or occupational exposure to lead, blood lead (PbB) determination is the most widely applied, both for defining the extent of exposure and for following individuals in known exposures. Other measurements can be useful in either high or low exposure situations to supply additional information. Free erythrocyte protoporphyrin (FEP) is a useful analyte in the screening of large numbers of children.[1,2] It is fast, inexpensive, free from most contamination problems and a sensitive indicator of moderate exposure to lead. It is most appropriate in establishing the existence of an exposure above that of the majority of the population. Once FEP is elevated it remains so for a long time. This is advantageous in identifying children who may have had an exposure to lead which has permanently or temporarily ceased prior to screening. However, it severely limits FEP as a practical method for following individuals on a regular basis.

Inhibition of the enzyme delta-aminolevulinic acid dehydratase (ALAD) is an even more sensitive marker for lead exposure. Several authors[3,4] have presented data which suggest that this enzyme is partially inhibited even by the level of lead exposure experienced by much of the urban population. A finding of either high FEP or high ALAD calls for further investigation and a PbB determination.

Where higher exposure is suspected, the amount of delta-aminolevulinic acid or coproporphyrin excreted in the urine (ALAU and CPU) are both useful. But CPU is not specific for lead poisoning and neither ALAU nor CPU are consistently elevated except in severe plumbism.[5] Therefore, despite its shortcomings elaborated elsewhere,[6] PbB determination remains the most widely applicable and accepted method for monitoring lead exposure.

PbB determinations have been carried out for many years.[7] Despite the long history, a regular complaint among analytical chemists has been the relatively poor interlaboratory agreement of all the available analytical methods. Several previous reports[8-12] of interlaboratory comparative studies and one monograph[13] have served to document the problem. In addition, because of the current emphasis on PbB by the various regulatory agencies, the problem is readily apparent to those interested.[14-16] We report here a new interlaboratory proficiency study of PbB determinations which indicates that some improvement has occured in recent years.

Although several of the previous studies[9,11,12] did use human blood collected from individuals occupationally exposed to lead, others used spiked blood. Current proficiency programs run by the Center for Disease Control (CDC) and

TABLE I
Results from all Laboratories

Blood Pool	N	Mean	S.D.	Intralaboratory Data	
				N	CV
IV low	16	48.6	5.9	16	3.0%
high	16	56.9	5.1	17	7.2
V low	18	40.7	5.4	8	3.2
medium	18	57.5	6.7	8	1.7
high	18	74.2	7.3	8	2.8
VI low	22	38.9	4.6	8	6.3
medium	22	55.0	6.5	8	3.2
high	22	63.0	5.0	8	3.1
VII low	23	25.0	6.2	9	10.7
medium	23	41.0	5.5	9	4.6
high	23	57.9	6.5	9	4.3
VIII low	37	36.4	7.8	9	4.8
high	37	70.1	9.0	9	2.7
IX low	38	43.4	5.6	12	4.3
high	38	68.5	7.5	12	5.5

The results of one participating laboratory have been omitted from this tabulation. They were continuously erratic.

various states generally use animal blood. While these samples are useful, the ideal sample remains human blood in which lead is incorporated into the red cell by "normal" processes. Thus, when this program was undertaken, it recognized that no suitable human blood standard was available that compared in quality to the various standard reference materials manufactured by the National Bureau of Standards.

This laboratory performs approximately 30,000 PbB determinations annually on venous blood samples obtained primarily from workers in the lead industry. The portion of each sample remaining after the determination is normally collected into six pools characterized by varying lead concentration. Cut points are set at 20, 40, 60, 80 and 100 micrograms of lead per deciliter of blood (μg/dL). The pooling has been done in order to provide a suitable supply of quality control samples. In the fall of 1974 the decision was made to offer portions of these pools to other laboratories for an interlaboratory comparison of PbB determinations.

procedure

Blood samples which arrive in this laboratory generally are already hemolyzed. If the sample has not for some reason been collected in a tube containing Triton X-100, then this hemolyzing agent is added immediately upon receipt of the sample. Hemolyzed blood remaining after analysis is pooled daily and frozen. This is accomplished by identifying one liter, acid washed, linear polyethylene bottles to serve for each pool.

When needed for this study, each pool was thawed and filtered through a heavy layer of gauze using only gravity in order to avoid frothing. Each pool was then gently swirled in a large Erlenmeyer flask to provide homogeneity. After the pool was well mixed, it was divided into 10 mL portions in screw-top, acid washed, linear polyethylene vials and refrozen.

Each pool was analyzed numerous times in this laboratory over a period of two weeks prior to shipment in order to assure homogeneity. Data summarizing the intralaboratory coefficient of variation (CV) for each pool are included in Table I.

Finally, samples were mailed to the participating laboratories in insulated containers with enough dry ice to insure frozen delivery in two days. Generally, this is sufficient. However, each laboratory was asked to describe the condition of each sample when received. During the course of the study there has been no clear evidence to indicate sample degradation

TABLE II
Results from
AIHA Approved Laboratories

Blood Pool	N	Mean	S.D.
IV low	11	46.5	4.9
high	11	54.8	3.6
V low	13	39.2	4.7
medium	13	56.3	7.1
high	13	72.8	8.1
VI low	15	38.8	4.3
medium	15	54.3	6.6
high	15	62.3	5.1
VII low	15	25.3	7.4
medium	15	40.3	6.1
high	15	57.3	7.2
VIII low	20	38.5	8.9
high	20	69.5	9.4
IX low	19	42.7	4.0
high	19	66.9	6.8

The results of one participating laboratory have been omitted from this tabulation. They were continuously erratic.

when the sample has been thawed upon arrival due to extended delivery time.

results

This interlaboratory program has gone through nine rounds as of the end of 1977. Some problems during the first year limited the usefulness of the available data. These included poor sample packaging in one round, inferior sample containers in another round and a small number of participants in the initial rounds. During the past two years these typical start-up problems have been eliminated. This report presents data obtained in the six rounds of 1976 and 1977.

Table I is a summation of results from the participating laboratories. In three of the rounds (IV, VIII and IX) only high and low specimens were sent out. In the remaining rounds (V-VIII) medium range specimens accompanied the high and low ones.

Although it was suspected that the data for all laboratories might fit a log-normal rather than a normal distribution, this was not the case. For all blood pools the geometric and arithmetic means were virtually identical.

Included in Table II are the means and standard deviations of those laboratories in the study which have been accredited for lead determination by the American Industrial Hygiene Association (AIHA) as of February, 1978. These laboratories must successfully determine lead in air filters sent regularly by AIHA. While the air filter is a significantly less complex matrix than blood, the accreditation does indicate proficiency in lead determination.

Along with the data, the laboratories identified the analytical method used in each round. Table III lists the data subdivided by method. The most popular single procedure was the Delves cup microanalytical method, although there is some indication that its popularity is waning. This was followed by the extraction procedure using ammonium pyrollidine dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK). These two procedures combined with flameless atomic absorption made atomic absorption the overwhelming favorite. Other methods used by several laboratories include the classic dithizone extraction, anodic stripping voltammetry and emission spectrography.

Where more than one or two laboratories had used a particular procedure, there was no significant bias which could be attributed to the method of analysis. Thus, from this data one must draw the conclusion that the three atomic absorption methods, the dithizone extraction and anodic stripping voltammetry are equally accurate. Precision of analysis depends more on the individual laboratory than the method of analysis. Precision will be discussed below.

discussion

The CDC, in its PbB proficiency programs, has established ad hoc guidelines for determining an acceptable range of reported results. This range is defined as ± 15% around the mean of PbB values greater than 40 μg/dL, and ±6 μg/dL for PbB values of 40 μg/dL or less.

Using these guidelines, it was possible to identify those laboratories which performed consistently well throughout their participation in the program. Twelve laboratories were so identified retrospectively using the following criteria:

1. the laboratory must have participated in at least the last three rounds (one year).
2. the laboratory must have had zero or one determination outside the acceptable range in the last six rounds (two years).

Of the twelve selected laboratories, nine had perfect records and three had each missed one of fifteen determinations.

This group is interesting for a number of reasons. First, these are laboratories which never differed markedly from the mean of the larger population; in addition, they agreed very consistently with each other. If it is assumed that there exist some laboratories which perform PbB determinations more accurately and more precisely than others for whatever reason, then it can be expected that the better laboratories should be identified by this type of process. The strength of this confidence rests on consistent performance over two years at various concentrations of lead. Second, if this process does select out the better laboratories, then it should be possible to estimate the optimum interlaboratory variation which can be expected

TABLE III
Results Subdivided by Method of Analysis

Blood Pool	Delves Cup			APDC-MIBK			Flameless AA		
	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
IV low	9	51	5	5	45	3			
high	9	62	7	5	53	4			
V low	10	43	5	5	37	3	2	45	1
medium	10	60	5	5	51	4	2	62	16
high	10	76	5	5	71	9	2	83	10
VI low	11	41	4	7	35	2	1	33	-
medium	11	57	6	7	51	3	1	38	-
high	11	64	6	7	62	3	1	51	-
VII low	13	24	4	7	24	6	2	23	2
medium	13	43	13	7	40	5	2	43	6
high	13	55	9	7	57	6	2	58	12
VIII low	12	34	3	11	35	14	8	41	12
high	12	66	5	11	72	18	8	70	21
IX low	10	43	3	12	43	6	9	45*	9
high	10	65	5	12	69	8	9	70*	11

Blood Pool	Dithizone			Anodic Stripping			Emission Spec.		
	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
IV low				1	46	-	1	58	-
high				1	57	-	1	54	-
V low				1	43	-	1	45	-
medium				1	60	-	1	68	-
high				1	81	-	1	76	-
VI low	1	40	-	1	38	-	1	46	-
medium	1	55	-	1	52	-	1	70	-
high	1	67	-	1	61	-	1	70	-
VII low	1	29	-	1	21	-	1	46	-
medium	1	51	-	1	38	-	1	52	-
high	1	63	-	1	62	-	1	72	-
VIII low	4	40	12	4	39	6	1	52	-
high	4	65	9	4	72	22	1	80	-
IX low	4	43	4	4	43	3			
high	4	71	9	4	70	5			

*Results from one laboratory (low = 200, high = 234) omitted to avoid total distortion of data.

at this time for PbB determination. Third, it might be interesting to identify the methods of analysis used by these successful laboratories.

Table IV contains means and standard deviations for the twelve selected laboratories. A set of CDC acceptable ranges was also calculated based only on these twelve laboratories. Two of these did not participate in rounds IV and V. In addition, the three data points which were not within the CDC acceptance ranges were not included in this tabulation. Thus, Table IV probably contains the best available estimates of the concentrations for these fifteen samples. Although in comparing Tables I and IV the means are not very different, the variances (equal to the square of the standard deviation) are much smaller for the data in Table IV. The average coefficient of variation (CV) in Table IV is about seven percent. This can be contrasted with a CV of an unselected group of laboratories of about fifteen percent.

In comparing the means of Tables I and IV, thirteen of fifteen means ($X^2 = 4.03$, $P < 0.05$) are higher in Table I. This suggests a common bias present in the results from the laboratories which perform less well. Contamination at the laboratory or during the determination and old standards which may have lost a portion of the analyte to the container walls are likely explanations. In examining all results from all

TABLE IV
Results from Twelve Selected Laboratories

Blood Pool	N	Mean	S.	CV	Revised CDC Range
IV low	10	47.2	2.5	5.2%	40.1 - 54.3
high	10	57.0	3.6	6.3	48.5 - 65.5
V low	10	39.8	2.9	7.2	33.8 - 45.8
medium	10	66.1	5.1	9.0	47.7 - 64.5
high	9*	75.7	2.9	3.8	64.4 - 87.0
VI low	11*	36.7	3.2	8.7	30.7 - 42.7
medium	12	53.2	3.0	5.6	45.3 - 61.2
high	12	62.1	3.5	5.6	51.8 - 70.4
VII low	12	23.1	3.1	13.4	17.1 - 29.1
medium	12	40.2	2.9	7.2	34.2 - 46.2
high	12	57.3	3.7	6.4	48.7 - 65.9
VIII low	12	34.5	3.0	7.4	28.5 - 40.5
high	12	67.8	3.3	4.8	57.7 - 77.9
IX low	11*	42.1	3.1	8.8	35.8 - 48.4
high	12	66.6	4.3	6.4	56.6 - 76.6

*Analyses in which one laboratory was outside the CDC acceptance range.

laboratories, high values are indeed more common than low values. However, extremely low reports did occur as well.

Of these twelve laboratories, six regularly use the Delves cup procedure, five use the APDC-MIBK extraction and one uses anodic stripping voltammetry. Most laboratories using flameless atomic absorption only participated in rounds VIII and IX and therefore were not eligible for inclusion in this selection.

Figures 1 through 4 chart the performance of four participating laboratories relative to the results of the twelve laboratories in Table IV. Data have been normalized to the CDC range using the formula:

$$\frac{\text{(individual result)} - \text{(mean of 12 selected labs)}}{\text{6; or 15\% of mean of 12 selected labs}}$$

Laboratory 1 is a highly respected institution which has performed quite adequately in all but one round. This one inaccurate performance emphasizes the importance of the extensive daily quality control procedures necessary to avoid occasional aberrant results. Laboratories 2 and 3 were regularly inaccurate, one high and the other low. It appears that their performances have been improving recently, perhaps as a result of participation in this and other proficiency programs. Note that laboratory 3 has switched from regularly low results for all levels of PbB to disturbingly high results for the low controls only.

Laboratory 4 is interesting because of its performance in relation to the method of analysis used. In rounds V through VII the Delves cup procedure was used. After initial problems, good results were obtained in round VII. Then the method of analysis was changed to flameless atomic absorption, and performance degenerated in round VIII only to improve somewhat, after a second apparent learning period, in round IX.

Figures 2-4 suggest the possibility of a learning process which has taken place as a result of proficiency testing. Among the twelve laboratories in Table IV one would not expect to find the effects of such a learning process; these laboratories have already achieved accurate and precise PbB analyses. Indeed the CV does not improve significantly with time. Among other laboratories there does appear to be a tendency, not statistically significant, to improve in ability to measure PbB with time.

Of the several previous interlaboratory evaluations of PbB published, one[9] was perhaps the most extensive and alarming. This study involved twelve blood samples in two rounds. The authors suggested three alternative methods for evaluating the performance of participating laboratories. The most attractive of the three was the regression of laboratory results on the true (substitute mean) values. Using this procedure they reported very disappointing results. Of 43 laboratories

In Figures 1 through 4 data for the high pools are represented by a triangle, data for the medium pools are represented by a square and data for the low pools are represented by a circle.

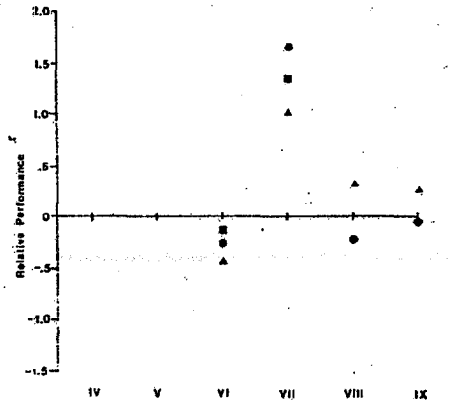

Figure 1 — This laboratory, while normally accurate, had one bad round.

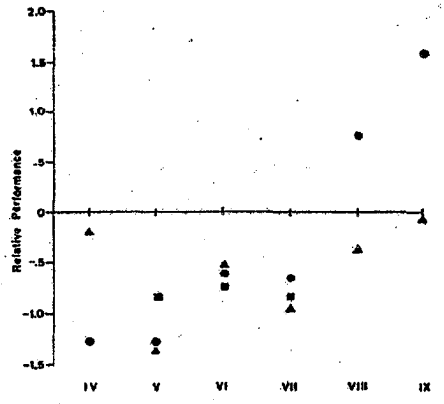

Figure 3 — This laboratory changed from regularly low results to somewhat more adequate performance.

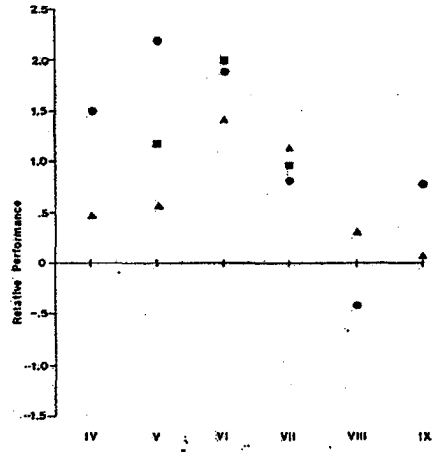

Figure 2 — This laboratory changed from regularly high results to adequate performance.

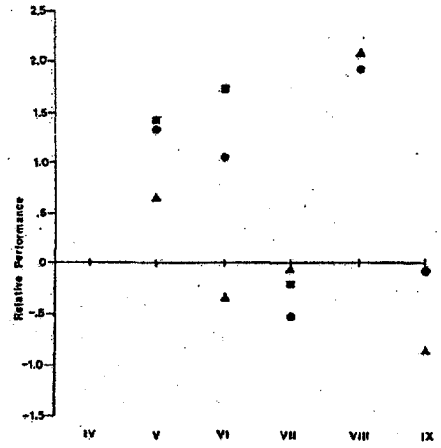

Figure 4 — After mastering one procedure, this laboratory switched its procedure with disappointing results.

participating in both rounds, only 18 had acceptable results in both (26 in the first and 36 in the second). Furthermore, of these 18 the slopes of the regression lines were significantly different in several cases for the two rounds. Acceptability was based arbitrarily on the correlation coefficient (r) $\geqslant$ 0.95 and the standard error of the estimate (S_{yx}) $\leqslant$ 15. Most of the laboratories which were not judged acceptable were characterized as having poor to negative correlation coefficients.

In order to compare the two studies, r and S_{yx} have been calculated for all 24 laboratories which participated in at least three rounds of the present study. See the appendix for the formulas used. Table V contains the results. Despite three more blood samples and the fact that samples from six different rounds were calculated together, each of which might be expected to introduce extra variability, similar percentages of laboratories (46% vs. 42%) meet the same criteria of acceptability in each study. In addition, all but one of 24 laboratories in the present study have a very high ($>$ +0.89) value for r. The range of the slope of the regression line was 0.78 - 1.34 in the present study. The earlier

TABLE V
Regression Results of Laboratory Data vs. Mean Values

Laboratory	N	Slope	Correlation Coefficient	$S_{y.x}$
1	15	1.01	0.99	5.5
2	15	1.14	0.99	7.9
3	15	1.04	0.99	4.7
4	10	0.96	0.98	11.5
5	15	1.03	0.94	28.9
6	15	0.87	0.92	29.2
7	13	1.11	0.90	74.7
8	15	1.34	0.31	3652.
9	15	1.01	0.99	3.2
10	15	0.97	0.98	10.4
11	15	1.00	0.99	4.1
12	15	0.90	0.99	4.5
13	15	1.02	0.95	22.9
14	15	0.90	0.92	29.5
15	15	1.11	0.99	6.5
16	15	1.09	0.94	33.0
17	15	0.80	0.91	27.4
18	10	0.99	1.00	0.3
19	10	0.94	0.95	24.1
20	10	1.04	0.96	24.0
21	7	1.08	0.90	91.6
22	13	0.78	0.89	34.0
23	13	1.09	0.96	21.3
24	11	0.97	0.95	11.9

study reported 14 of 36 slopes from their acceptable laboratories (two per laboratory) to be outside this range.

conclusions

During the last decade there has been some improvement in the ability of different laboratories to agree with each other on PbB determinations. With current methodologies, the optimum CV which can be expected for PbB is about 7%. It is reasonable to expect at least twice this value in any broad survey of laboratories.

It is more common to receive a falsely elevated PbB result, due most probably to a variety of causes including contamination at the sites of collection and analysis, or improperly maintained standards. However, artifically low results are occasionally reported as well.

There are some indications in this data set that participation in proficiency programs of this type does aid a laboratory in improving accuracy and precision of analysis.

appendix

$$r = b\sqrt{\frac{\Sigma(x-\bar{x})^2}{\Sigma(y-\bar{y})^2}}$$

$$S_{yx} = \frac{\Sigma(y-\bar{y})^2 - b^2\Sigma(x-\bar{x})^2}{n-2}$$

where: $$b = \frac{\Sigma xy - (\Sigma x \Sigma y)/n}{\Sigma(x^2) - (\Sigma x)^2/n}$$

$\bar{x}$ = average of "true" concentrations of all blood samples

$\bar{y}$ = average of observed concentrations of all blood samples

NB: the formula for r is not that reported by Keppler, *et al.*; however, it is the one which must be used to obtain the results in their Table XI.

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Refresher course schedule and registration procedure changes . . .

Two important modifications pertaining to the Refresher Courses, to be held in conjunction with the AIH Conference in Chicago, May 27-June 1, 1979, have been made.

Courses will be presented Sunday afternoon, May 27 from 1 to 5 P.M. and Monday afternoon, May 28 from 1 to 5 P.M. (instead of Sunday afternoon and Monday morning, as in the past and previously announced for 1979).

Attendance at any and all refresher course sessions must be signed up in advance, on a pre-registration basis. There will be NO at-conference registration for refresher courses. Be sure to plan ahead and pre-register.

Refresher course monitor volunteers needed . . .

Volunteers are needed to serve as monitors and assist instructors conducting refresher courses at the 1979 AIH Conference in Chicago. In addition to the satisfaction of being of service, a monitor benefit is free admission to the course monitored.

NOTE that the schedule has been changed from previous years and as initially announced for 1979. Monitors will serve on Sunday afternoon, May 27, from 1 to 5 P.M. and Monday afternoon, May 28, from 1 to 5 P.M. (NOT Monday Morning).

To volunteer, contact Sam Kaplan, Refresher Course Committee Chairman, Enviro-Control, Inc., One Central Plaza, 11300 Rockville Pike, Rockville, MD 20852 (301) 468-2500.