Quality Control in the Measurement of Blood Cholinesterase Activities Among Persons Exposed To Pesticides[1]

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Over the past several years considerable attention has been paid to the health of farm laborers and other personnel exposed to pesticides. Many attempts have been made to use infrequent or periodic blood cholinesterase activity measurements as a means of estimating adverse exposure to anticholinesteratic materials--attempts that, for a variety of reasons often fail to detect any altered status of pesticide workers' health. However, field studies, despite some pitfalls in experimental design, have shown conclusively that farm laborers do suffer depressed levels of cholinesterase activity incurred during the course of their normal employment. Among the most serious considerations in assessing the damage from pesticide exposure is the confidence that can be placed on laboratory cholinesterase activity measurements especially since a wide variety of laboratories with varying degrees of competence in this area are employed in the determinations. Some report values which are empirical; some report activity in terms of unit chemical change with time showing the actual quantities being measured. For all procedures so called "normal limits" are given which presumably apply to non-exposed populations. Acute changes in activity can be detected by any procedure, but more modest alterations can be confirmed only when the sensitivity of the method of assay, the precision and the degree of quality control used in making measurements are assessed fully.

When a single laboratory is involved in cholinesterase determinations results can be compared over a period of time if adequate means of intralaboratory quality control are used. Often, however, it is necessary to compare results on an interlaboratory basis and unless all laboratories involved are under the same regimen of control the measurements between them and over time become questionable, if not suspect. In the absence of interlaboratory quality control "normal limits" are, in essence, applicable only to the laboratory that established them. Since legal claims have arisen on the basis of reported cholinesterase activities that are just beneath the "normal limits" there is no point in using these criteria to define normality when their values are so uniquely

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established. Comparisons of intra- or interlaboratory determinations have been made to illustrate and confirm the need for a quality control program.

Methods and Results

To evidence the necessity of interlaboratory quality control we can first consider some results obtained in a single laboratory, by the same personnel, using reagents from the same source, and employing the same methods but with two different instruments of comparable function and manufacture. Enzyme activities for samples of bovine plasma cholinesterase were determined, with only slight modification in procedure, by the pH-stat technique reported by Nabb and Whitfield . Values determined with one pH-stat differed by more than ± 5 percent from those determined

TABLE 1

Interlaboratory Variation in Cholinesterase Assays of a Series of Human Blood Samples

Sample No.	Cholinesterase Activity (u moles/min/ml)					
	Plasma			RBC		
	Lab 1	Lab 2	Lab 1 / Lab 2	Lab 1	Lab 2	Lab 1 / Lab 2
1	4.24	5.77	0.73	11.9	13.1	0.91
2	2.98	3.88	0.77	11.9	14.6	0.82
3	3.04	4.50	0.68	12.5	12.8	0.98
4	2.90	4.11	0.71	10.5	14.1	0.74
5	4.37	4.81	0.91	13.2	16.4	0.80
6	3.08	4.20	0.73	9.9	14.2	0.70
7	4.31	5.77	0.75	11.7	18.0	0.65
8	4.85	6.52	0.74	10.5	13.8	0.76
9	5.59	8.33	0.67	10.3	12.8	0.80
10	3.24	3.88	0.84	10.7	14.2	0.75
11	4.74	5.84	0.81	13.4	16.5	0.81
12	6.58	8.76	0.75	13.0	14.9	0.87
13	6.25	8.14	0.77	14.4	12.7	1.13
14	1.25	1.67	0.75	11.8	12.6	0.94
15	6.72	8.70	0.77	10.1	11.6	0.87
16	4.41	5.80	0.76	12.2	12.8	0.95
17	2.96	4.19	0.71	12.4	16.5	0.75
18	3.03	4.15	0.73	10.1	11.6	0.87
19	2.76	3.73	0.74	14.0	16.1	0.87
20	4.66	6.21	0.75	11.7	14.6	0.80

with the other instrument in 19 of 31 pairs of measurements (61 percent). There were 8 values (26 percent) differing by more than ± 10 percent and 4 (13 percent) differing by more than ± 15 percent. In another series of 50 pairs of comparable assays on the same two instruments 40 percent differed by more than ± 5 percent, 18 percent of the paired values differed by more than ± 10 percent, while 10 percent differed by more than ± 15 percent. The sensitivity in routine cholinesterase activity determinations on a single instrument should allow no more than 5 percent variation and with some minor differences between instruments perhaps a combined variation of 7 to 9 percent can exist.

If the enzyme assays are performed on an interlaboratory basis and then compared, the problem is further compounded. When two different laboratories each using the pH-stat technique with instruments of the same manufacture assayed aliquots from a series of human blood samples some striking differences appeared (see Table 1). The magnitude of the differences between laboratories in assay values of a given sample, particularly with regard to plasma cholinesterase activity, was sufficient in many cases to evidence adverse exposure if the results on the same sample were, instead, to be considered sequential determinations on the same subject measured in laboratory 2 prior to, and laboratory 1 following exposure. Both laboratories were well experienced in the techniques of assay and both were consistent with a high degree of precision.

To exemplify further the variation in assay values of cholinesterase (bovine serum), this time among several laboratories

TABLE 2

Interlaboratory Variation in Cholinesterase Assays of a Series of Human Serum Samples

Laboratory	Cholinesterase Activity (u moles/min/ml)			
	Sample 1	Sample 2	Sample 3	Sample 4
A	2.7 , 2.7	- -	- -	2.5 , 2.5
B	2.26, 2.27	2.19, 2.24	- -	2.24, 2.24
C	2.5 , 2.5	- -	- -	2.2 , 2.2
D	3.66, 3.52	2.90, 3.03	- -	- -
E	2.83, 2.83	2.57, 2.57	2.70, 2.73	2.40, 2.44
F	3.14, 3.10	2.81, 2.84	3.23, 3.07	2.84, 2.84
G	3.78, 3.78	2.99, 2.84	2.99, 3.14	3.10, 3.10
H	3.40, 3.38	3.26, 3.20	3.41, 3.35	2.90, 2.90
I	2.7 , 2.8	- -	- -	3.0 , 2.8
J	2.02, 2.17	1.88, 1.92	2.01, 2.10	- -
K	- -	2.68, 2.68	2.83, 2.90	2.53, 2.59
L	- -	2.15, 2.20	2.45, 2.55	- -

all using the same method (pH-stat) and exhibiting good precision, we need only examine the data of Table 2.

The difference between maximum and minimum activities measured for anyone of these four samples--47, 42, 41 and 29 percent of the maximum values, respectively--is sufficient to establish cholinesterase inhibition were it not known that the laboratories were assaying portions of the same sample in each of the four sets of measurements. Generally, laboratories were precise and consistent in reporting high or low values for each sample assayed. For any other methods of cholinesterase analysis similar variations are fully expected.

Discussion

Due to the range in analytical results between laboratories even if each used the same methods of analysis, it becomes senseless for each to use the same "normal limits" as a criterion in delineating the absence or degree of adverse exposure to pesticides. Either each laboratory must establish with great care, the sensitivity of measurements by the particular method employed, together with their own realistic normal limits; or alternatively, all laboratories should be guided by a single quality control program. Maintenance of the program must be the responsibility of a central agency and laboratory. As is not uncommon in establishing quality control regulations, this laboratory would supply control samples whose cholinesterase activity is known, at least approximately, but reported as an absolute value to be used by all participating laboratories. In effect, all instruments are then calibrated to the same reference point. Each laboratory would assay the reference sample along with specimens whose values are to be determined and against the stated absolute cholinesterase activity of the reference sample all other values can be calculated. Then, and only then, can meaningful normal limits be set which apply to all laboratories and cholinesterase levels can be compared between the different laboratories, different instruments of similar function and with time.

Literature Cited

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