Radioimmunoassay

Practical Program for Statistical Evaluation of Normal Range Data*

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A program utilizing three methods of normal range estimation for radioassay is described. The methods are chosen for their variable dependence upon test sample characteristics such as size of population sampled, skewing, and spread of values T_3 uptake, T_4 CPB, TSH, B_{12}, and folate normal specimens are screened using the standard deviation, data point ranking, and percentile interpolation methods. While three of the five test populations are normally distributed, two are best evaluated by methods other than the standard deviation. The folate test population is positively skewed and the data point ranking method provides the most valid estimate. The test population for the B_{12} assay is small (32) and the spread between values large; therefore, the percentile interpolation method provides the most valid normal range estimate.

Normal range estimates for clinical testing are an integral part of the clinical laboratory's responsibility. Although published ranges can be generally adopted, they are often not satisfactory in accounting for particular laboratory conditions and geographical location of the population.

Several techniques have been published describing statistical handling of normal data *(1,2)*. The more sophisticated techniques require involved data reduction and large populations which most clinical laboratories are not equipped to handle. Furthermore, they are not universally applicable since each technique is influenced by characteristics of the sample population such as skewing, shape of normal curve, and number of samples.

In an attempt to establish a simple but valid procedure for evaluating normal data for all radioassay testing, a program has been developed which employs three techniques chosen for their simplicity and variable dependence upon these test sample characteristics. By evaluating the data according to these three techniques, the characteristics of the population are described and a more valid judgment may be made concerning the most applicable method providing the most realistic range. The application of this program is illustrated for T_3 uptake, T_4 CPB, TSH, B_{12}, and folate assays.

Methods

Sera for this study were obtained from blood bank donors, and thereby considered normal.

The mean, standard deviation (sd) and normal range are evaluated using the equation

$$sd = \sqrt{\frac{\Sigma(\bar{x}-x)^2}{N-1}}$$

Two standard deviations equal to 95% confidence limits are used throughout. While two times the sd only approaches 95% confidence limits as the sample size approaches 120, these are the two commonly employed limits and as such are compared here directly. The data point ranking method requires ordering the data points in an increasing value sequence *(3)*. Five percent of the total number of data points is determined and discarded equally from the high and low ends of the rank. For example, if the total number of data points collected is 92, then 2 of those points would be discarded from the low end and 2 from the high end. The 95% confidence limits normal range in this case is quoted between the third lowest and third highest data points (Table 1).

By grouping the ranked data points in dose increments (Table 2), a graphical display of the data point ranking method can be produced. The number of data points falling within a specified dose increment is plotted versus dose on a linear scale (Fig. 1). The resulting histogram does not produce a normal range but defines skewing, its extent and direction.

The third method of normal range estimate is a nonparametric method independent of distribution of

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TABLE 1. Ordered Data Points for T_4 CPB (n=92)

3.8	6.7	7.9 (Mean)	9.2
4.2	6.7		9.2
	6.7	8.0	9.3
4.5	6.8	8.2	9.3
4.6	6.8	8.2	9.4
5.2	6.8	8.2	9.5
5.2	6.9	8.3	9.5
5.3	6.9	8.3	9.6
5.3	7.0	8.3	9.8
5.3	7.1	8.4	10.1
5.4	7.1	8.4	10.2
5.6	7.1	8.4	10.2
5.8	7.2	8.4	10.5
5.8	7.2	8.4	10.6
5.8	7.2	8.5	10.8
5.9	7.3	8.6	10.9
6.0	7.3	8.6	11.0
6.0	7.4	8.7	11.0
6.2	7.6	8.7	11.2
6.3	7.6	8.7	11.5
6.4	7.6	8.8	12.2
6.4	7.7	8.8	
6.5		8.9	12.6
6.7	7.8 (Median)	8.9	12.6

normal population *(4)*. Cumulative frequencies are calculated from ranked data points by the equation

$$\frac{\text{sequence number of data point}}{\text{total number of data points}} \times 100.$$

In this way each data point is assigned a percentile (Table 3). These percentiles are plotted on probability paper versus dose and the best fit straight line is drawn (Fig. 2). The normal range of the 95% confidence limits is determined from this plot by interpolation of the line at 2.5 and 97.5% to the corresponding dose.

In all sampled populations, data points are excluded if the difference in dose between the data point and the next highest or lowest value is equal to or greater than one-third of the total range of values *(5)*.

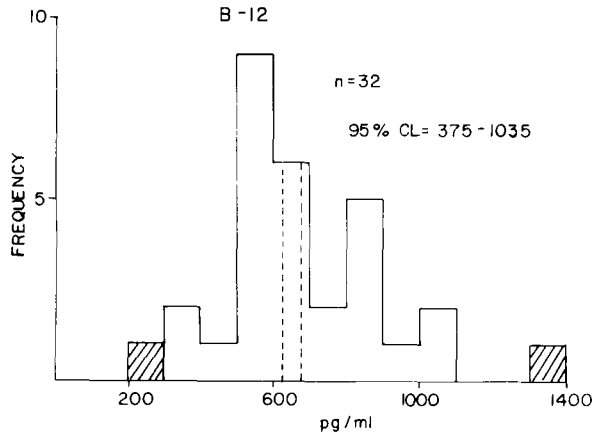

FIG. 1. B_{12} histogram of 32 normal specimens.

TABLE 2. Histogram Data for B_{12}

pg/ml	Dose range	Frequency
200	200-300	1
375	300-400	2
474	400-500	1
512		
517		
523		
530		
545	500-600	9
559		
567		
574		
575		
620		
624		
625		
664		
668	600-700	7
675		
695		
702		
710	700-800	3
715		
802		
816		
818	800-900	5
841		
858		
968	900-1000	1
1002		
1035	1000-1100	2
	1100-1200	0
	1200-1300	0
1312	1300-1400	1

Results and Discussion

The sd while convenient and mathematically accurate, assumes that the sample population forms a normal bell-shaped curve with 95% of the values evenly distributed about the mean (Fig. 3). In the case of the T_4 CPB assay, the normal range by the sd method would appear to be valid. However, the folate normal population is not evenly distributed about the mean since the sd yields a range cutoff of −0.2 on the low end (Table 3). This would indicate positive skewing causing a large sd which cannot be applied evenly about the mean. The histogram of the

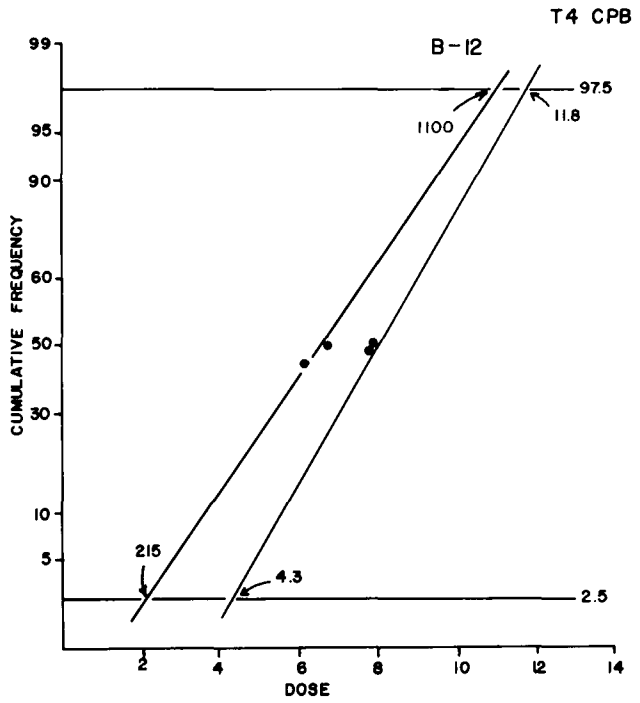

FIG. 2. Percentile interpolation of normal range estimate for B_{12} and T_4 CPB.

T_4 CPB data confirms the facsimile of a bell-shaped curve (Fig. 4). As would be expected, the normal range determined by the data point ranking method compares well with that determined by the sd method for this assay (Table 3).

The folate histogram, on the other hand, supports the suspicion of positive skewing (Fig. 5). The mean and median are not comparable and the data point ranking range is quite different from that determined by the sd method (Table 3). The data point ranking method is very dependent upon individual values at the high and low ends of the normal population.

In the B_{12} assay, only 32 normal specimens were collected and a considerable amount of spread between values is apparent on the high and low ends of the range (Table 4). When 95% confidence limits are applied to the assay (discarding one value from each end of the range), the 200 pg/ml data point falls below 95% of the values and the next highest data point is 375 pg/ml. While the low cutoff by the data point ranking method is 375, the actual low cutoff may be some where between 200 and 375 pg/ml.

TABLE 3. Normal Range Estimates

Assay		sd	Data point ranking	Percentile interpolation
T_3 uptake	99	31.2-51.6	31.6-50.4	31.0-47.0
T_4CPB	92	4.1-11.7	4.5-12.2	4.3-11.8
TSH	99	1.0 - 4.5	1.0 - 3.8	1.0 - 4.4
B_{12}	32	236-1105	375-1035	215-1100
Folate	78	-0.2 - 9.8	1.4 - 9.8	—

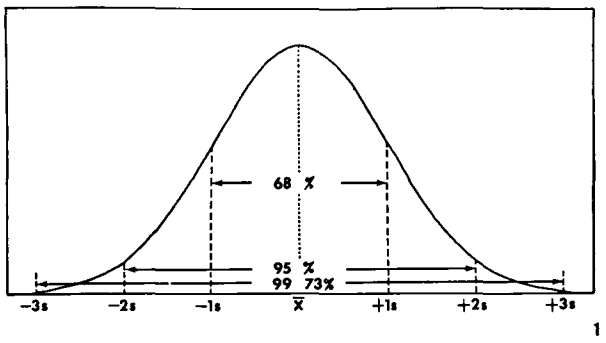

FIG. 3. Normal bell-shaped curve; 95% of total values within 2 sd.

Percentile intepolation can be used in such cases to generate a more valid cutoff independent of any individual data points. If the population is distributed according to a bell-shaped curve as in the case of the T_4 CPB assay, these cumulative frequencies will form a straight line. The folate data do not conform well to the percentile method since the plotted cumulative frequencies do not fall on a straight line (Fig. 6). A curve can be drawn, but the error introduced in drawing such a curve must be considered.

The T_4 CPB percentile interpolated normal range compares well with those estimated using both the sd and data point ranking methods. The B_{12} range, however, has

TABLE 4. PERCENTILE CALCULATION FOR B_{12}

Data point	pg/ml	Percentile
1	200	3.13
2	375	6.26
3	375	9.30
4	474	12.50
5	512	15.63
6	517	18.75
7	523	21.88
8	530	25.00
9	545	28.13
10	559	31.25
11	567	34.38
12	574	37.50
13	575	40.63
14	620	43.73
15	624	46.88
16	625	50.00
17	664	20.75
18	668	20.88
19	675	59.38
20	695	62.50
21	702	65.63
22	710	68.75
23	715	71.88
24	802	75.00
25	816	78.13
26	818	81.25
27	841	84.38
28	858	26.81
29	968	90.63
30	1002	93.75
31	1035	96.88
32	1312	100.00

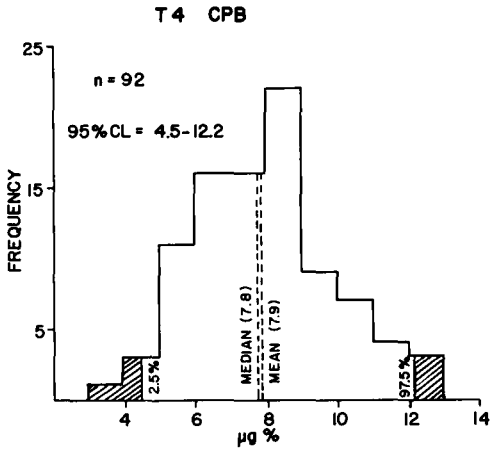

FIG. 4. T4 CPB histogram of 92 normal specimens; bell-shaped population.

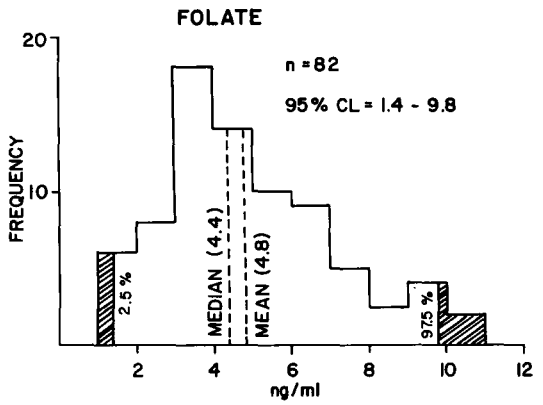

FIG. 5. Folate histogram of 82 normal specimens illustrates positive skewing.

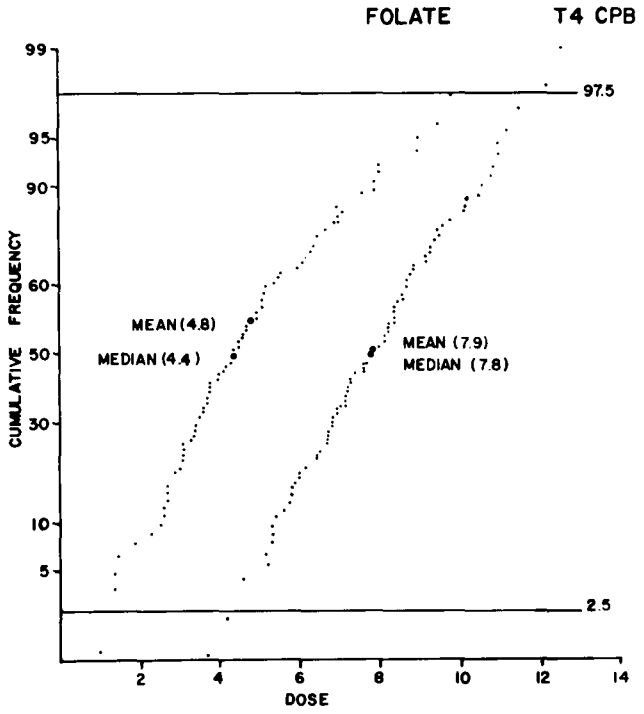

FIG. 6. Percentile interpolation plot of cumulative frequencies versus dose for folate and T4 CPB normal values.

a low cutoff of 215, which indeed falls between the 200 and 375 seen with the data point ranking method (Fig. 2).

In finally selecting a normal range estimate from the methods applied to each test population certain priorities are in order. The sd method is the most mathematically accurate but only applies when the data conform to a bell-shaped curve and the sample size is large enough to warrant statistical validity. Percentile interpolation is the second method of choice since it is independent of individual values if the percentile-versus-dose plot conforms to a straight line. The data point ranking normal range is used whenever the sd and percentile interpolation methods do not apply, but is applicable only where skewing or spread of values is minimal and sample size is reasonably large.

In the case of the T_3 uptake, T_4 CPB, and TSH, the sd is used since the data have been proven to be normally distributed about the mean, and indeed normal range estimates by all three methods for each assay are comparable (Table 3).

The small number of samples in the B_{12} assay precludes valid use of the sd method and the spread of values at the extreme ends of the normal population invalidates the data point ranking method, leaving the percentile interpolation method, the most acceptable. The sd method could not be applied to the folate data since the normal population sampled was proven to be non-Gaussian and the percentile ranking method did not result in a straight line fit; therefore, the data point ranking normal range estimate is more valid for the folate assay (Table 3).

The advantage of using three methods for determination of normal range estimates is obvious in two of the assays tested. Furthermore, in the case of the folate data, the common sd method clearly cannot be applied. If all normal data are routinely run through these three simple test methods, more valuable normal range estimates can be determined.

It is perhaps noteworthy that the normal range which can be generated in a routine clinical laboratory is often based upon less than a sufficient number of specimens. If normal ranges have been generated using large populations (>220) and are published, they can be confirmed in a particular laboratory using the preceding outlined methods.

References

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