

# THE GRAPHICAL EVALUATION OF RESULTS OF SIMPLE AND MULTIPLE SLOPE-RATIO ASSAYS

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GRAPHICAL methods for the analysis of results of parallel-line quantitative assays have been presented by several writers, for example<sup>1,2,3,4</sup>. The statistical analyses of results of slope-ratio assays, based on a linear relationship between the response and the dose itself, are generally more tedious than for parallel-line assays, particularly when more than one test preparation is investigated at a time. Hence a method for rapid computation of such assays by means of nomograms may prove especially useful for routine work.

A simplified procedure for computation of slope-ratio assays of any number of preparations<sup>5</sup> has been used to develop the nomograms to be described here. In each of the examples to be presented there are a common zero dose and two non-zero doses of each test preparation, with doses evenly spaced; this is the most economic arrangement of doses which will allow tests of validity, and these examples have been chosen as being of the most useful type for routine assays. The nomograms used to estimate the relative potencies are independent of the number of replications used, but for finding the fiducial limits fresh charts are needed for different numbers of replications. A brief description of the construction of charts for assays with more dose-levels, or without a common zero dose, will also be given.

The range has been used to estimate the error variance, with a consequent further saving in computation, and the only calculations necessary involve some addition and subtraction, and a few divisions which may conveniently be done on a slide rule.

Throughout, the notation will be that used in the earlier paper<sup>5</sup> but all symbols will be explained here for completeness.

## EXAMPLES

### *Simple assay (one test preparation)*

Table I shows a computing sheet for a 5-point assay of one test preparation with four replications. The common zero dose and two non-zero doses of each preparation are evenly spaced, and the upper level of each preparation is taken as one unit.

The total and range for each treatment are entered on the sheet, the ranges are totalled to give the value  $r$ , and the treatment totals are used as shown in the table to give the values  $Q$  and  $H$  which are themselves totalled to give  $S(Q)$  and  $S(H)$ . The values of  $Q_r/Q_s$  and  $Q_s/r$  are calculated and noted (a slide rule gives sufficient accuracy).

The tests of validity described by Finney<sup>6</sup> as "blanks" and "intersections" are made by examining the values of  $H$ . The numerical values of

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TABLE I  
COMPUTING SHEET FOR SIMPLE SLOPE-RATIO ASSAY

Preparation Dose (units)	Control 0	Standard 1		Test 1		Total
Response ..	1.3	3.6	6.1	3.2	4.7	—
	1.7	3.6	6.3	3.2	4.9	—
	1.5	3.7	6.0	3.1	4.5	—
	1.4	4.1	6.3	2.9	5.0	—
Total ..	$T_0$ 5.9	$S_1$ 15.0	$S_2$ 24.7	$T_1$ 12.4	$T_2$ 19.1	77.1 = $G$
Range ..	0.4	0.5	0.3	0.3	0.5	2.0 = $r$
<i>Validity tests</i>						
$H = 2S_1 - S_2$ , etc.		5.3		5.7		11.0 = $S(H)$
		$S(H) - 2T_0 = -0.8$		$t_1r = 3.10$ (5% level)		
		$H_S - H_T = -0.4$		$t_2r = 2.62$ (5% level)		
<i>Estimation of relative potency and fiducial limits</i>						
$Q = 5(S_1 + 2S_2) - 3G$ , etc.		90.7		21.7		
$Q_T/Q_S$ .. .. .		—		0.239		
$Q_S/r$ .. .. .		45.35		—		
$R$ (relative potency) ..		—		0.71		
$R_L, R_U$ (5 per cent. fiducial limits) .. .. .		—		0.66, 0.76		
Calculated values:						
Relative potency .. .. .				0.707		
5 per cent. fiducial limits .. .. .				0.658, 0.755		

$S(H) - 2T_0$  and  $H_S - H_T$  should not exceed  $t_1r$  and  $t_2r$  respectively, where  $t_1$  and  $t_2$  for the 5 per cent. and 1 per cent. levels of significance are shown in Table II.

If the validity tests are satisfied, the chart in Figure 1 is then used to estimate the relative potency and its fiducial limits. The points corresponding to  $Q_T/Q_S$  are found on the curves  $A_1A_1'$ ,  $A_2A_2'$  and joined. The intersection of this line with the vertical axis  $BB'$  gives the value of  $R$ , the

TABLE II  
VALUES OF  $t_1$  AND  $t_2$  FOR SIGNIFICANCE TESTS IN A SIMPLE 5-POINT SLOPE-RATIO ASSAY WITH  $n$  REPLICATIONS

$n$	$t_1$		$t_2$	
	5 per cent.	1 per cent.	5 per cent.	1 per cent.
2	2.41	3.78	2.04	3.20
3	1.71	2.43	1.44	2.05
4	1.55	2.14	1.31	1.81
5	1.50	2.05	1.27	1.73
6	1.49	2.02	1.26	1.70

relative potency. The point corresponding to  $Q_S/r$  is then located on the horizontal axis  $CC'$  and joined, in turn, to each of the two  $Q_T/Q_S$  points; the points of intersection of these lines, produced if necessary, with the vertical axis  $BB'$  give the 5 per cent. fiducial limits ( $R_L$  and  $R_U$ ).

A scale for  $R$  may be put on either of the curves  $A_1A_1'$ ,  $A_2A_2'$ , in which case the first operation may be avoided, but as the vertical scale for  $R$  is identical with that for  $R_L$  and  $R_U$ , and since both  $Q_T/Q_S$  points must be located, the first method avoids some detailed scaling.

In the example,  $Q_T/Q_S = 0.239$  and, using the chart in Figure 1, on joining the points found on  $A_1A_1'$  and  $A_2A_2'$ , a value of 0.71 for  $R$  is found on  $BB'$ . The point  $Q_S/r = 45.35$  on  $CC'$  is joined to the points  $Q_T/Q_S = 0.239$  on  $A_1A_1'$  and  $A_2A_2'$ , and the intersections of these lines with  $BB'$  give values of  $R_L$  and  $R_U$  equal to 0.66 and 0.76 respectively. These results compare favourably with the calculated values ( $R = 0.707$ ;  $R_L = 0.658$ ;  $R_U = 0.755$ ).

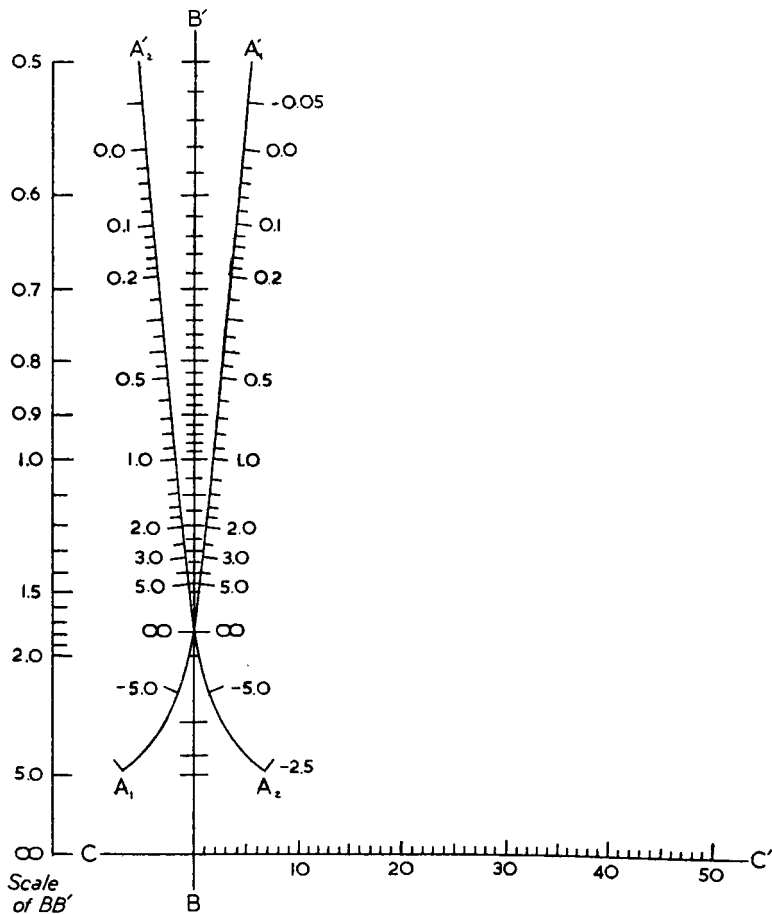


FIG. 1. Nomogram for simple 5-point assay with 4 replications.

- (i) Join  $Q_T/Q_S$  on  $A_1A_1'$  to  $Q_T/Q_S$  on  $A_2A_2'$  and read  $R$  on  $BB'$ .
- (ii) Join  $|Q_S|/r$  on  $CC'$  to  $Q_T/Q_S$  on  $A_1A_1'$  and  $A_2A_2'$  in turn and read  $R_L, R_U$  on  $CC'$ .

*Multiple assay (several test preparations)*

The example given here is an assay of three test preparations using a  $\{1 + 4(2)\}$ -point design, with a common zero dose and two levels of each of the four preparations; there are two replications of each treatment.

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TABLE III  
COMPUTING SHEET FOR A MULTIPLE SLOPE-RATIO ASSAY

Preparation Dose (units)	Control 0	Standard 1		Test (1)		Test (2)		Test (3)		Total
		$\frac{1}{2}$	1	$\frac{1}{2}$	1	$\frac{1}{2}$	1	$\frac{1}{2}$	1	
Response ..	1.10 1.40	4.06 4.23	7.20 6.90	4.05 3.80	6.94 6.40	3.25 3.18	5.10 5.20	3.60 3.60	5.75 5.79	— —
Total ..	$T_0$ 2.50	$S_1$ 8.29	$S_2$ 14.10	$T_{11}$ 7.85	$T_{12}$ 13.34	$T_{21}$ 6.43	$T_{22}$ 10.30	$T_{31}$ 7.20	$T_{32}$ 11.45	81.46 = $G$
Range ..	0.30	0.17	0.30	0.25	0.54	0.07	0.10	0.00	0.05	1.78 = $r$
<i>Validity tests</i>										
$H = 2S_1 - S_2$ , etc. ..		2.48		2.36		2.56		2.95		10.35 = $S(H)$
		$S(H) - 4T_0 = 0.35$		$t_1 r = 3.36$ (5 per cent. level)		$t_3 r = 2.10$ (5 per cent. level)				
		Range of $H = 0.59$								
<i>Estimation of relative potencies and fiducial limits</i>										
$Q = 9(S_1 + 2S_2) - 3G$										
etc. ..		84.03		66.39		— 1.11		26.52		175.83 = $S(Q)$
$S(Q)/Q$ ..		2.09		2.65		— 158.4		6.63		—
$bs$ ..		5.75		—		—		—		—
$bs/r$ ..		3.23		—		—		—		—
$R$ (relative potency) ..		—		0.93		0.67		0.78		—
$RL, RV$ (5 per cent. fiducial limits) ..		—		0.87, 0.99		0.62, 0.73		0.72, 0.84		—
Calculated values:—										
Relative potency ..				0.932		0.672		0.779		
5 per cent. fiducial limits ..				0.871, 0.993		0.617, 0.728		0.721, 0.836		

Treatment totals and ranges are entered and combined as shown in Table III. The range of the values of  $H$  is noted.

For validity,  $S(H) - 4T_0$  should not numerically exceed  $t_1 r$ , and the range of  $H$  should not exceed  $t_3 r$ , where  $t_1$  and  $t_3$  for different significance levels are shown in Table IV. These tests correspond respectively to the tests for blanks and intersections described by Clarke<sup>5</sup>.

TABLE IV  
VALUES OF  $t_1$  AND  $t_3$  FOR SIGNIFICANCE TESTS IN A MULTIPLE  $\{1 \div 4(2)\}$ -POINT SLOPE-RATIO ASSAY WITH  $n$  REPLICATIONS

$n$	$t_1$		$t_3$	
	5 per cent.	1 per cent.	5 per cent.	1 per cent.
2	1.89	2.72	1.18	1.51
3	1.43	1.96	0.93	1.17
4	1.33	1.79	0.87	1.09
5	1.30	1.74	0.86	1.07
6	1.30	1.73	0.86	1.07

If the assay appears to be valid, the relative potencies are found from Figure 2 as follows. The points corresponding to  $S(Q)/Q_s$  and  $S(Q)/Q_T$  are found on the lines  $OO'$  and  $P_1P_1'$  respectively and joined; the intersection of this line with  $P_2P_2'$  gives the value of  $R$ , the relative potency.

Thus in the example,  $S(Q)/Q_s = 2.09$ , and for the first test preparation  $S(Q)/Q_T = 2.65$ . A line joining the points corresponding to these values gives an estimate of the relative potency as 0.93 (the value obtained by calculation is 0.932).

To find the fiducial limits of the relative potencies, it is first necessary

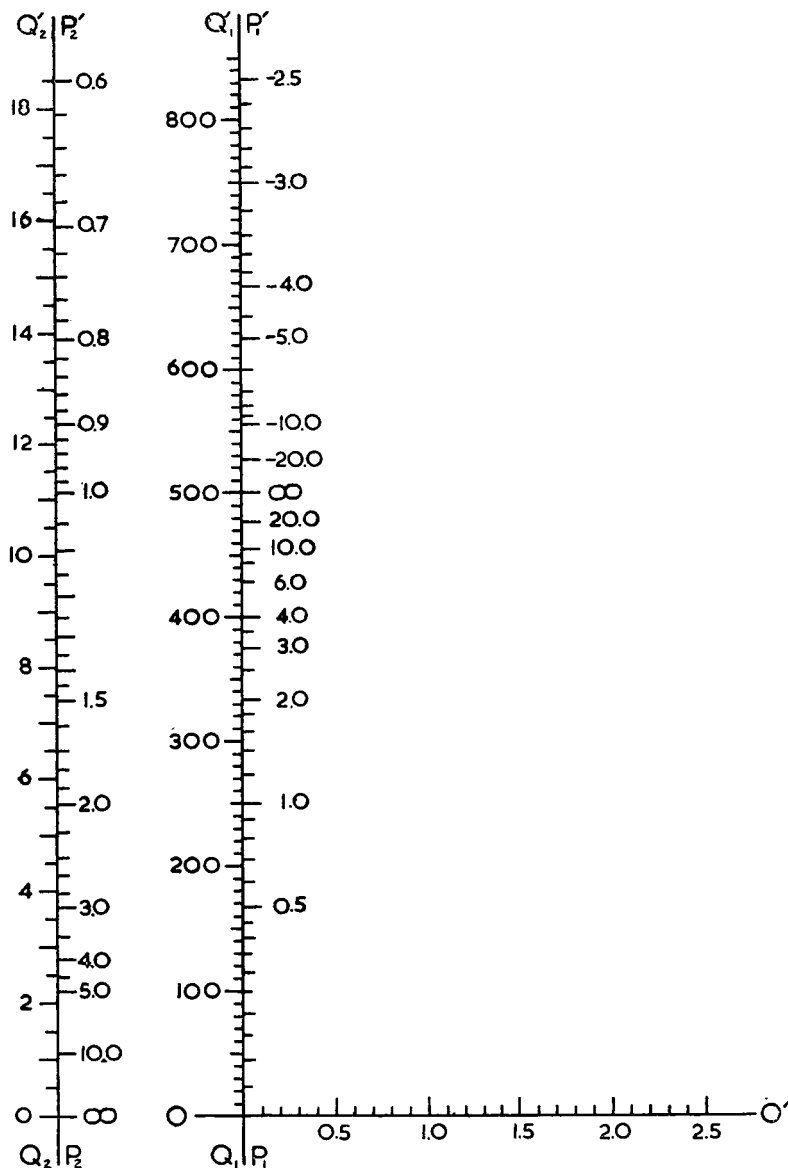


FIG. 2. Nomogram for multiple  $1 + 4(2)$ -point assay with 2 replications—I.

- (i) Join  $S(Q)/Q_s$  on  $OO'$  to  $S(Q)/Q_T$  on  $P_1P_1'$  and read  $R$  on  $P_2P_2'$ .
- (ii) Join  $S(Q)/Q_s$  on  $OO'$  to  $|S(Q)|$  on  $Q_1Q_1'$  and read  $|b_s|$  on  $Q_2Q_2'$ .

to estimate  $|b_s|$ , the numerical value of the slope of the dose-response line for the standard preparation. This is also obtained from Figure 2, by joining the points corresponding to  $S(Q)/Q_s$  and  $|S(Q)|$ , located on  $OO'$  and  $Q_1Q_1'$  respectively, and reading off the value of  $|b_s|$  given by the

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intersection of this line with  $Q_2Q_2'$ . In this example  $|b_s|$  is found to be about 5.75 (calculated value 5.775).

The chart in Figure 3 is then used to find the 5 per cent. fiducial limits of the relative potencies. The values of  $|b_s|/r$  is calculated as  $5.75/1.78$ , i.e., 3.23, and the corresponding point on the horizontal axis  $XX'$  is joined, in turn, to the points on the curves  $Y_1Y_1'$  and  $Y_2Y_2'$  corresponding to the relative potency of any one test preparation. The intersections of these two lines with the vertical axis  $ZZ'$  give the upper and lower fiducial limits. For the first test preparation in this example the 5 per cent. fiducial limits are found to be 0.87 and 1.00 (calculated values 0.871 and 0.993). The corresponding values for the other two test preparations may be rapidly read off in the same way.

### CONSTRUCTION OF THE CHARTS

The nomogram for the simple slope-ratio assay is derived from the relations

$$R = \{1 + J Q_T/Q_S\} / \{J + Q_T/Q_S\}$$

and  $R_L, R_U = R \pm K d_n^{-1} t (r/Q_S) (J - R) [\gamma(R - 1)^2 + 2R]^{\frac{1}{2}}$ ,

where  $\gamma = (5k^2 + 5k + 2)/2 (k^2 + k + 1)$ ,

$$J = \gamma/(\gamma - 1),$$

$$K = \frac{1}{(J + 1)} \sqrt{\frac{nk(k+1)(2k+1)}{6}},$$

$k$  is the number of non-zero dose-levels of each preparation,  $n$  is the number of replications of each treatment and  $d_n$ , the factor used in estimating the standard error from the range, is given in Table V.

TABLE V  
VALUES OF  $d$  AND  $v^*$

$i$	$d_i$	$v_i$
2	1.128	7.27
3	1.693	10.95
4	2.059	14.49
5	2.326	17.86
6	2.534	21.08
7	2.704	24.11
8	2.847	27.02
9	2.970	29.82

\* The above values, constants of the range distribution, have been obtained from tables given by Cox<sup>7</sup> based on data published by Pearson<sup>8</sup>.

A suitable nomogram for solving these equations is obtained by first drawing rectangular coordinate axes, marking off on the  $x$ -axis a scale for  $|Q_S|/r$  such that  $x = \mu |Q_S|/r$ , and on the  $y$ -axis marking off a scale for  $R_L$  and  $R_U$  such that  $R_L, R_U = \delta/y$ , where  $\delta$  and  $\mu$  are any convenient constants. The curves for  $R$  are then given by the equations

$$x = \pm \mu K d_n^{-1} t (J - R) [\gamma(R - 1)^2 + 2R]^{\frac{1}{2}}/R, \quad y = \delta/R,$$

where the value of  $t$  for the appropriate significance level is obtained from standard tables, entering the tables with  $(2k + 1)(n - 1)$  degrees of freedom. As explained in the example, it is convenient to mark off the

curves only with scales for  $Q_T/Q_S$  given by  $y = \delta(J + Q_T/Q_S)/(1 + JQ_T/Q_S)$ , and to use the  $y$ -axis to read off values of  $R$ .

For a simple slope-ratio assay without a zero dose, the procedure is just the same, except that  $\gamma$  is now equal to  $(5k + 1)/2(k - 1)$ ,  $Q$  is given by, for example,  $Q_S = 4(S_1 + 2S_2 + \dots + kS_k) - (k + 1)G$ ,  $K$  is replaced by  $L$ , where

$$L = \frac{1}{(J + 1)} \sqrt{\frac{2n(k + 1)(2k + 1)}{3k}}$$

and the  $t$ -table is entered with  $2k(n - 1)$  degrees of freedom.

When more than one test preparation is investigated at a time, the corresponding expressions for the relative potency and the fiducial limits are  $R = \{\gamma - 1 + Q_T/S(Q)\}/\{\gamma - 1 + Q_S/S(Q)\}$

$$\text{and } R_L, R_U = R \pm \frac{t}{(vk + 1)d_n} \left(\frac{r}{b_s}\right) \sqrt{\frac{6k[\gamma(R - 1)^2 + 2R]}{n(k + 1)(2k + 1)}}$$

where  $\gamma = \frac{vk(k - 1) + (3k + 1)(k + 2)}{vk(k - 1) + 2(2k + 1)}$ ,  $v$  is the number of preparations including the standard,  $t$  is found from tables with  $(vk + 1)(n - 1)$  degrees of freedom, and

$$b_s = 6[Q_S + (\gamma - 1)S(Q)]/n(k + 1)(2k + 1)(vk + 1).$$

These relations lead to three nomograms of which two can be drawn on the same chart. To find the value of  $R$  and of  $|b_s|$ , rectangular coordinate axes are drawn as in Figure 2. The  $x$ -axis is marked off to give values of  $S(Q)/Q_S$  using the linear scale  $x = \eta(\gamma - 1)S(Q)/Q_S$ , where  $\eta$  is a convenient scaling constant. The  $y$ -axis is marked off with two scales: one, linear, for  $S(Q)$  given by

$$y = 6\theta(\gamma - 1)|S(Q)|/n(k + 1)(2k + 1)(vk + 1)$$

and the other, non-linear, for  $S(Q)/Q_T$  given by

$$y = \phi[(\gamma - 1)S(Q)/Q_T]/[1 + (\gamma - 1)S(Q)/Q_T],$$

$\theta$  and  $\phi$  being further constants chosen for convenience. Another vertical axis is drawn at  $x = -\eta$  and marked off with a linear scale for  $b_s$  given by  $y = \theta|b_s|$ , and an inverse scale for  $R$  given by  $y = \phi/R$ .

The nomogram for finding the fiducial limits of each value of  $R$  is exemplified in Figure 3. The scale for  $|b_s|/r$  on the  $x$ -axis is given by  $x = \mu|b_s|/r$ , and that for  $R_L, R_U$  on the  $y$ -axis by  $R_L, R_U = \delta/y$ . The equations of the curves for  $R$  are

$$x = \pm \frac{\mu t}{(vk + 1)d_n} \sqrt{\frac{6k[\gamma(R - 1)^2 + 2R]}{n(k + 1)(2k + 1)R^2}}, y = \frac{\delta}{R}$$

so that the vertical scale for  $R$  is the same as that for the fiducial limits.

For an assay without a common zero dose, the charts are similar, but  $\gamma$  is then equal to  $[v(k - 1) + 3(k + 1)]/v(k - 1)$ ,  $Q$  is given by, for example,  $Q_S = 2v(S_1 + 2S_2 + \dots + kS_k) - (k + 1)G$ , the scale for  $S(Q)$  is  $y = 3\theta|S(Q)|(\gamma - 1)/n v(k + 1)(2k + 1)$ , the equation of the curve for  $R$  has  $vk$  instead of  $(vk + 1)$  in the denominator of the expression for  $x$ , and the  $t$ -table is entered with  $vk(n - 1)$  degrees of freedom.

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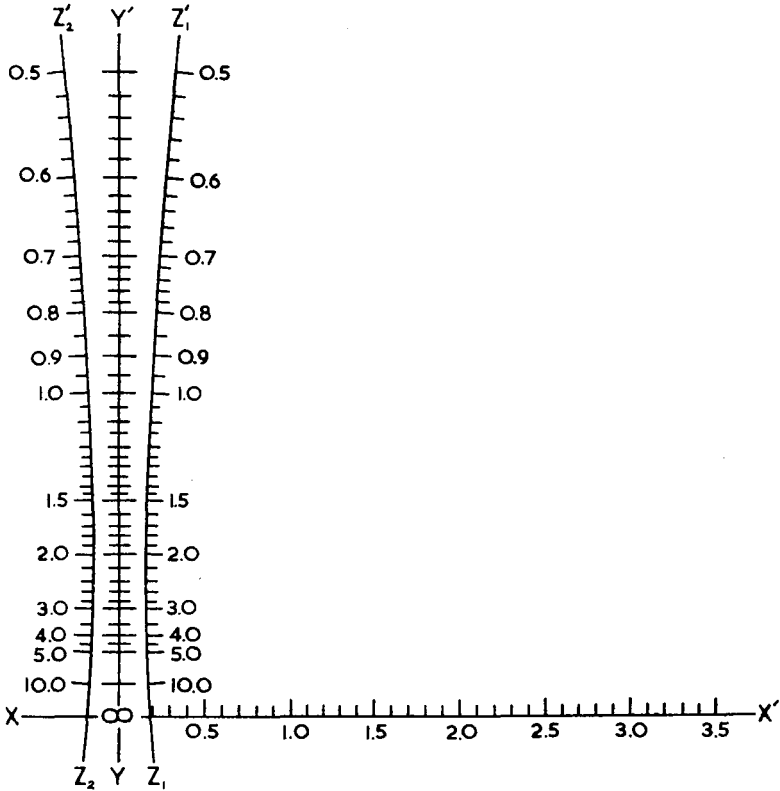


FIG. 3. Nomogram for multiple  $\{1 + 4(2)\}_1$ -point assay with 2 replications—II. Join  $|b_s|/r$  on  $XX'$  to  $R$  on  $Z_1Z'_1$  and  $Z_2Z'_2$  in turn and read  $R_L, R_U$  on  $YY'$ .

VALIDITY TESTS

The first step in the general application of validity tests as used in the above examples is the calculation of values of  $H$  where

$$H_s = 2(k - 1)S_1 + [2(k - 1) - 3]S_2 + [2(k - 1) - 6]S_3 + \dots - (k - 1)S_k,$$

and so on.

The test for "blanks" involves calculating the quantity  $S(H) - vk(k - 1)T_0/2$ , and comparing it with  $t_1r$ , where

$$t_1 = \frac{tk}{2(vk + 1)d_n} \sqrt{\frac{3vn(k^2 - 1)}{\gamma - 1}},$$

$d_n$  is given in Table V and  $t$  is found

from standard tables with  $(vk + 1)(n - 1)$  degrees of freedom. No test for "blanks" is made if there is no common zero dose.

In the case of a simple assay with a common zero dose the test for "intersections" is made by comparing the difference between the values of  $H$  for the two preparations with  $t_2r$ , where  $t_2 = t\sqrt{nk(k - 1)/(2k + 1)}/d_n$  and  $t$  has  $(2k + 1)(n - 1)$  degrees of freedom; when there is no common zero dose  $t_2 = t\sqrt{n(k - 1)(2k + 1)/k2d_n}$  and  $t$  has  $2k(n - 1)$  degrees of freedom.



For a multiple assay, the corresponding test for "intersections" may be made using a range test described by Cox<sup>7</sup>. When there is a common zero dose the range of the values of  $H$  should not be greater than  $t_3 r$  where  $t_3 = d_v F_{v_1, v_2} \sqrt{nk(k-1)(2k+1)/2}/(vk+1)d_n$ .  $F$  is found from variance ratio tables with  $v_1$  and  $v_2$  degrees of freedom, where  $v_1 = v_v$  and  $v_2 = (vk+1)v_n$ , using the values of  $v$  given in Table V. When there is no common zero dose,  $t_3 = d_v F_{v_1, v_2} \sqrt{n(k-1)(2k+1)/2k}/v d_n$ ,  $v_1 = v_v$  and  $v_2 = vkv_n$ .

If  $k$  is greater than 2, further tests of validity may be made by examining the deviations from linearity for the responses to non-zero doses; such tests are easily effected by using the usual orthogonal coefficients for the treatment totals (see, for example<sup>9,10</sup>). Thus for  $k = 3$ , the quantities  $C_s = S_1 - 2S_2 + S_3$ , etc., are computed for each preparation. The deviation from linearity for any one preparation will be significant if the corresponding value of  $C$  is greater than  $t_4 r$ , where  $t_4 = t \sqrt{6n}/(vk+1)d_n$ , and  $t$  is found for  $(3v+1)(n-1)$  or  $3v(n-1)$  degrees of freedom, depending on whether there is or is not a common zero dose. Alternatively an overall test of curvature may be made by examining whether the total of the values of  $C$  exceeds  $\sqrt{k} t_4 r$ . It is unlikely that in routine assays  $k$  will exceed 3, and consequently further tests for  $k > 3$  will not be given here.

#### DISCUSSION

Charts such as those presented here considerably reduce the time required for evaluation of the estimates of relative potencies and their fiducial limits in slope-ratio assays, and should prove particularly useful for multiple assays, which are common in microbiological work. Once the experimental design has been decided on, the charts are drawn up and may be used repeatedly.

The use of range in place of standard deviation cuts down the necessary computing, but does not give the best estimate of error except when  $n = 2$ , and may introduce a bias in the estimates of fiducial limits if the responses are not normally distributed. When  $n$  is small, however, these limitations are unlikely to be of practical importance. The procedure described in this paper for estimating the fiducial limits also applies only when the observations are not arranged in blocks. A further qualification of the method is that the fiducial limits are obtained from the approximate variance of  $R$ , applicable only when the assay is of good precision (see, for example<sup>9,10</sup>); this condition should, however, be aimed at before an assay method is adopted for routine use and, in any case, holds for most microbiological assays. The estimates of relative potency obtained from the chart are unaffected by these considerations.

#### SUMMARY

Charts are presented and described for the rapid evaluation of results of simple and multiple slope-ratio assays with and without a common zero dose.

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