A SIMPLE HAND-HELD CALCULATOR PROGRAM FOR PROCESSING DENGUE VIRUS PLAQUE REDUCTION NEUTRALIZING ANTIBODY TITER DATA

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BACKGROUND : Since the introduction of the plaque reduction neutralizing antibody test in 1966, the standard SEATO Lab/AFRIMS protocol for processing raw plaque counts has been :

1. Calculate the % (avg plaque count test/avg plaque count control).
2. Plot the % values on probit paper vs the log of the serum dilution.
3. By eye, draw a best-fit line through the data points.
4. Record the titer at which the line intersects the 50% value as the $PRNT_{50}$.

The rapidly developing hand-held calculator industry has recently introduced several relatively inexpensive programmable computer-calculators, complete with pre-programmed statistical routines and branching capability, which are capable of performing all the functions necessary to compute $PRNT_{50}$'s directly from plaque counts.

METHODS : To accurately process raw plaque counts into a $PRNT_{50}$'s three basic operations must be performed.

1. Transform % to probits. The probit value for any % can be closely approximated as follows (from quotation 26.2.22 in Handbook of Mathematical Functions, National Bureau of Standards Applied Math Series #65, 1964) :

If % $\leq$50, compute

(a) $t = \sqrt{-2 \ln (\%) + 9.21034}$

(b) $Z = t - \dfrac{2.30753 + 0.27061t}{1 + 0.99229t + 0.04481t\gamma}$

(c) Probit = 5-z

If % >50, first set % = 100-%, then compute

(a) t = as above

(b) Z = as above

(c) Probit = 5 + Z

2. Perform linear regression analysis of plot of $\log_2 \frac{(\text{dilution N})}{10}$ vs probit value of $\frac{(\text{Avg. plaques/well (dilution N)}}{\text{Avg. plaque/well (control)}} \times 100\%$ to determine y intercept (b) and slope (m) of best least squares fit line.

3. Calculate titer as :

$$PRNT_{50} = 10 \times 2^{\frac{(5-b)}{m}}$$

A simple program was developed for processing raw plaque counts into a $PRNT_{50}$ value using a Texas Instrument-59 programmable calculator. After the calculator is programmed the operator need only be concerned with the following steps :

Enter	Press	Print
Avg. plaques/well control	A	Avg. plaques/well control
Avg. plaques/well 1/10 dilution of serum	B	Probit value of % plaques/well (1/10 dilution) Plaques/well (control)
Avg. plaques/well for 1/40 dilution	C	Probit value for 1/40 dilution
Avg. plaques/well for 1/160 dilution	D	Probit value for 1/160 dilution
Avg. plaques/well for 1/640 dilution	E	Probit value for 1/640 dilution
	A	$PRNT_{90}$ from least squares fit
		$PRNT_{50}$ from least squares fit ("4 point method")
		$PRNT_{50}$ estimated using % reduction at a 1:40 dilution only ("one point method")*
		Slope of least squares fit line
		Correlation coefficient of least squares fit line

* assumes $\frac{\Delta \text{ (Probits)}}{\Delta \left(\log_2 \frac{\text{dilution N}}{10}\right)} = 0.4$

RESULTS : Data from the last 99 unknown virus identification assays were reviewed. Using raw plaque data which had been obtained by standard methods with reference rhesus monkey anti-sera to dengue 1-4, the following methods of data analysis were compared :

(a) $PRNT_{50}$ obtained by standard method by eye vs the $PRNT_{50}$ obtained by the "4 point program method."

(b) $PRNT_{50}$ by the "4 point program method" vs $PRNT_{50}$ estimated from the percent reduction of plaques obtained at only a 1:40 dilution of anti-sera "1 point program method".

"Eye" vs "4 point program" methods :

A comparison of the $PRNT_{50}$'s previously derived by the "eye" method with $PRNT_{50}$'s calculated by the "4 point program method" using data of neutralization of D2 viruses by anti-D2 reference anti-serum is presented in Figure 1 as an example. A correlation coefficient of 0.976 is obtained. Table 1 presents a summary of similar comparisons of "eye" vs "4 point program" $PRNT_{50}$'s for all 16 combinations of neutralization of dengue virus types by reference anti-dengue sera. There is very close agreement.

"4point program" vs "1 point program" methods :

Sample comparisons of $PRNT_{50}$'s obtained by least squares fit analysis of data from all four serum dilutions versus the $PRNT_{50}$ obtained using data from only the percent neutralization by serum at a 1:40 dilution, for the neutralization of D2V by anti-D1 and anti-D2 are presented in Figure 2A and 2B. Good agreements are obtained (the "1 point program" method tends to over-estimate the titer when the average number of plaques at 1:40 is less than 1). Table 2 presents a summary of similar comparisons of "4 point program" vs "1 point program" $PRNT_{50}$'s for all 16 combinations of neutralization of dengue virus types by reference anti-dengue sera.

The validity of using the value of 0.4 as the average slope

$$\Delta \text{ probits} \div \Delta \log_2 \left[\frac{\text{Dilution N}}{10}\right]$$

was assessed by averaging the actual slopes found by the least squares fit analysis using all 4 points. The average slope is in fact close to 0.4 for all virus-anti-sera combinations (Table 3).

Identification of unknowns : comparison of accuracy of the "4 point program" versus the "1 point program" methods :

As shown in Figure 3, usually there is no ambiguity of virus type when unknowns are identified by neutralization with the "4 point program" method; average homologous titers are 10 to 100 times greater than average heterologous titers. However, some virus isolates are more readily neutralized by heterologous anti-sera; occasionally the ratio of the $PRNT_{50}$ by the homologous (most reactive) reference anti-sera is only 2 to 4 times greater than the highest $PRNT_{50}$ obtained with heterologous reference anti-sera. However, no more ambiguity occurs with the "1 point program" method than with the "4 point program" method (Figure 4): 7/97 (7%) versus 6/97 (6%) had homologous/highest heterologous titer ratios which could be considered in the "ambiguous range" (4).

CONCLUSION : Based on these analyses, we now type all unknown isolates by recording the % plaque reduction obtained with a 1:40 dilution of reference anti-serum, and estimating the $PRNT_{50}$ with each anti-serum by the "one point program method". When ambiguous results occur, the typing procedure is repeated by using the "4 point program" method.

Table 1. X = Log_{10} (Eye $PRNT_{50}$), Y = Log_{10} (4 point-program $PRNT_{50}$)

Virus	Anti-sera	N	X + S.D.*	Y + S.D.	r
D1**	D1	2	3.14	3.09	
	2	2	ND	1.00	
	3	2	1.27	1.27	
	4	2	ND	1.00	
D2	D1	70	1.512 ± .327	1.496 ± .335	.985
	2	67	2.830 ± .365	2.863 ± .404	.976
	3	70	1.402 ± .373	1.396 ± .382	.989
	4	70	1.331 ± .308	1.333 ± .311	.924
D3	D1	19	1.543 ± .322	1.500 ± .328	.915
	2	18	1.057 ± .165	1.042 ± .132	.987
	3	20	2.912 ± .251	2.964 ± .264	.921
	4	17	1.082 ± .176	1.062 ± .161	.957
D4	D1	5	1.212 ± .333	1.186 ± .316	.992
	2	5	1.060 ± .134	1.046 ± .103	1.000
	3	5	1.252 ± .346	1.222 ± .304	1.000
	4	5	2.544 ± .275	2.556 ± .318	.970

* Titers less than 1:10 scored as 1:10, log = 1.0

** Standard deviations and correlation coefficients not calculated for D1 (N = 2)

Table 2. X = Log_{10} (4 point-program $PRNT_{50}$), Y = Log_{10} (1 point-program $PRNT_{50}$)

Virus	Anti-sera	N	X + S.D.*	Y + S.D.	r
D1**	D1	2	3.08	3.07	-
	2	2	1.00	1.00	-
	3	2	1.27	1.35	-
	4	2	1.00	1.00	-
D2	D1	70	1.496 ± .335	1.594 ± .480	.984
	2	71	2.910 ± .440	3.099 ± .365	.805
	3	70	1.382 ± .382	1.414 ± .434	.903
	4	70	1.333 ± .311	1.297 ± .331	.910
D3	D1	20	1.494 ± .320	1.539 ± .328	.880
	2	18	1.042 ± .137	1.076 ± .138	.612
	3	21	2.987 ± .279	3.265 ± .512	.432
	4	17	1.062 ± .161	1.069 ± .165	.938
D4	D1	5	1.186 ± .316	1.174 ± .389	.962
	2	5	1.046 ± .103	1.000 ± .000	1.000
	3	5	1.222 ± .304	1.226 ± .311	.999
	4	5	2.556 ± .318	2.944 ± .558	.921

* Titers less than 1:10 scored as 1:10; log = 1.0

** Stand deviations and correlation coefficients not calculated for D1 (N = 2)

Table 3. $X = \text{Log}_{10}$ (4 point-program $PRNT_{50}$), Y = Slope of $\frac{\Delta \text{ Profit}}{\Delta \log_2(\frac{\text{dilution}}{10})}$

Virus	Anti-sera	N	X + S.D.*	Y + S.D.	r
D1**	D1	2	3.08	.438	
	2	2	0.39	.242	
	3	2	1.27	.356	
	4	2	0.35	.197	
D2	D1	70	1.431 ± .473	.479 ± .162	.634
	2	71	2.910 ± .440	.467 ± .091	-.745
	3	70	1.254 ± .583	.415 ± .176	.339
	4	70	1.179 ± .552	.477 ± .202	.269
D3	D1	20	1.470 ± .349	.432 ± .156	.492
	2	18	0.608 ± .436	.320 ± .138	.616
	3	21	2.987 ± .279	.455 ± .085	-.492
	4	16	0.621 ± .508	.270 ± .102	.373
D4	D1	5	0.838 ± .663	.315 ± .202	.977
	2	5	0.662 ± .442	.267 ± .158	.892
	3	5	0.922 ± .663	.334 ± .249	.908
	4	5	2.556 ± .318	.528 ± .074	.160

* Titers less than 1:10 scored as actual derived value

** Standard deviations and correlation coefficients not calculated for D1 (N = 2)

Figure 1.

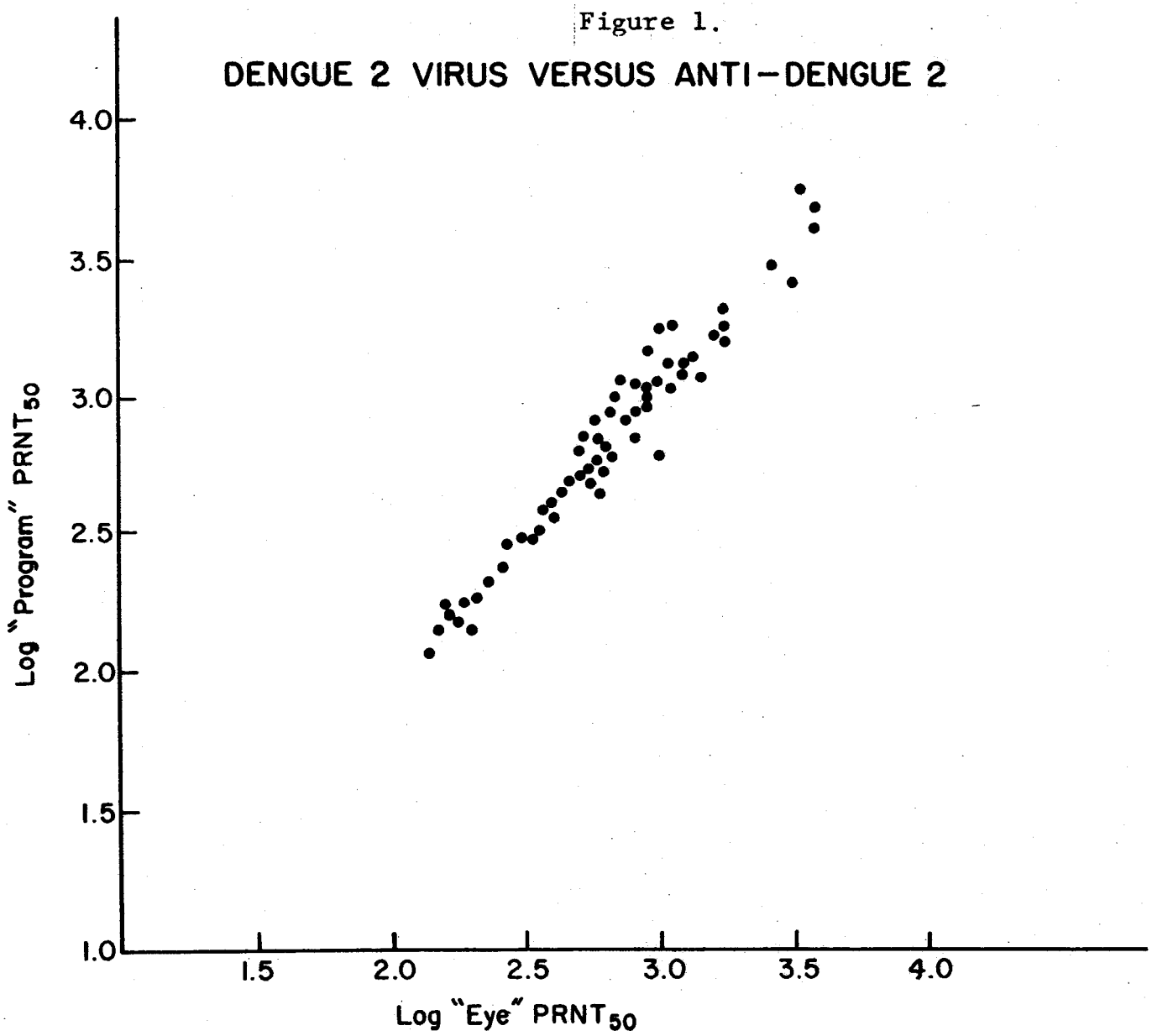

DENGUE 2 VIRUS VERSUS ANTI-DENGUE 2
Log "Program" PRNT50
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Log "Eye" PRNT50

Figure 2A.

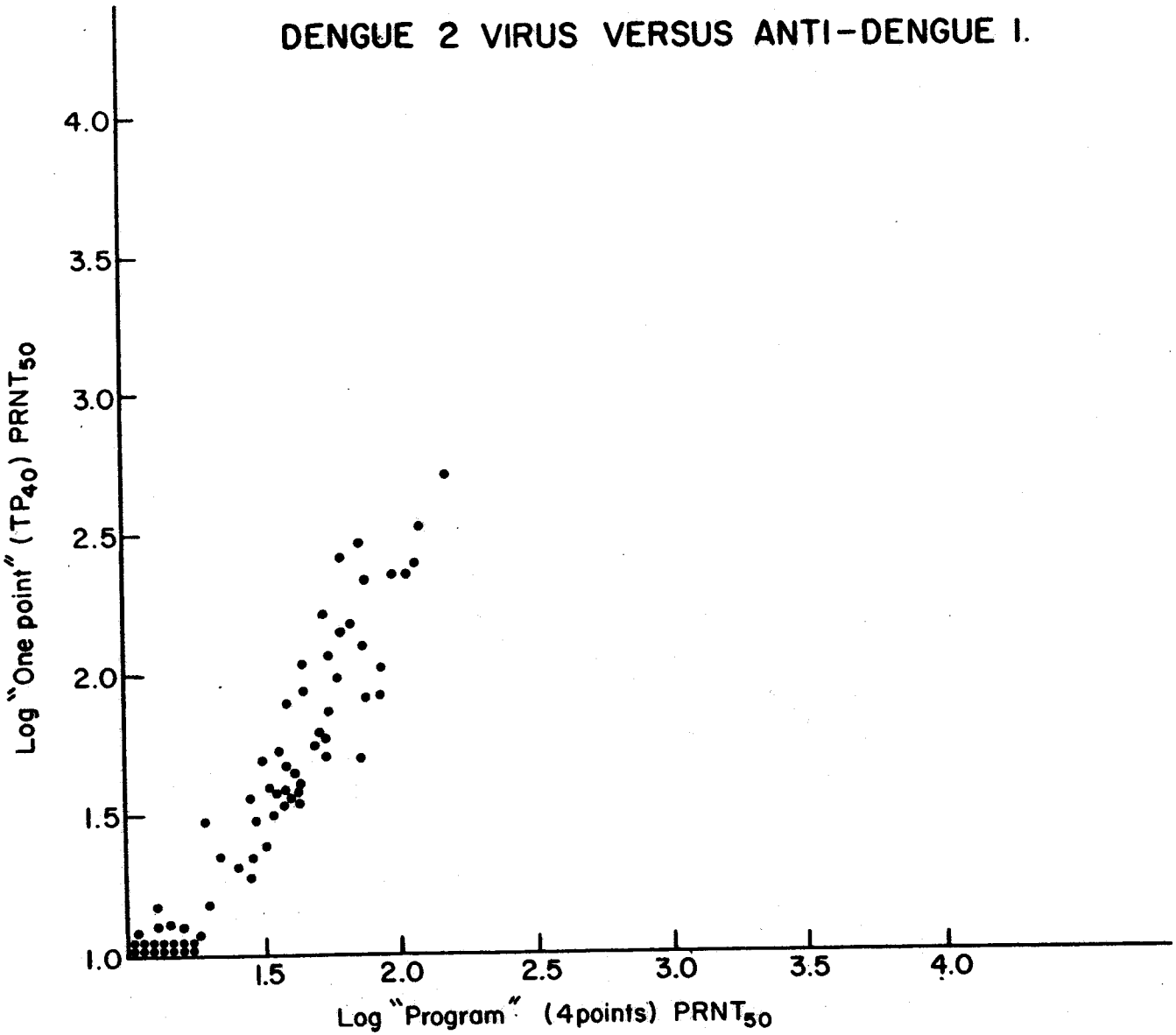

DENGUE 2 VIRUS VERSUS ANTI-DENGUE I.
Log "One point" (TP40) PRNT50
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Log "Program" (4points) PRNT50

Figure 2B.

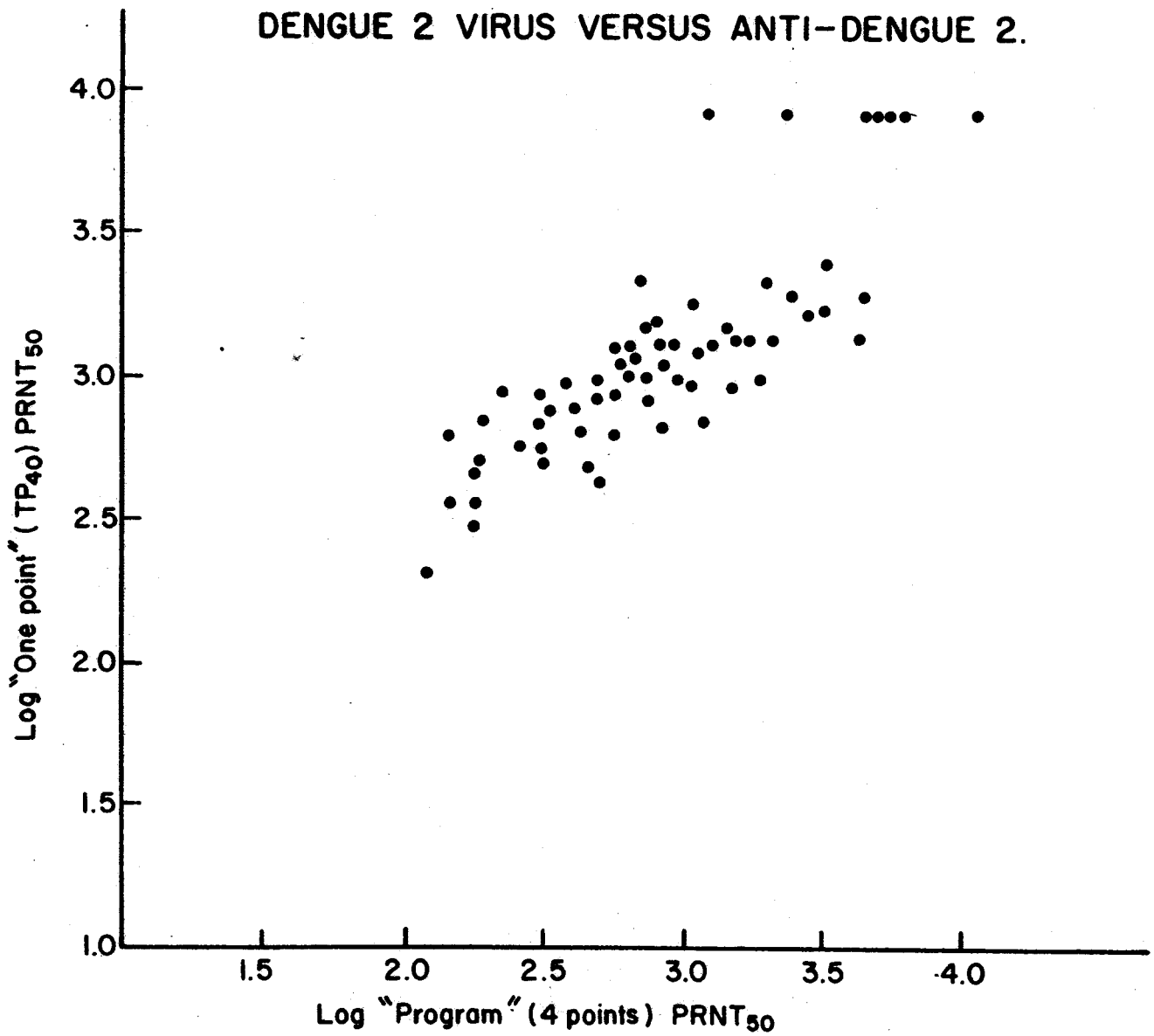

DENGUE 2 VIRUS VERSUS ANTI-DENGUE 2.
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Log "One point" (TP40) PRNT50
1.5
2.0
2.5
3.0
3.5
4.0
Log "Program" (4 points) PRNT50

Figure 3.

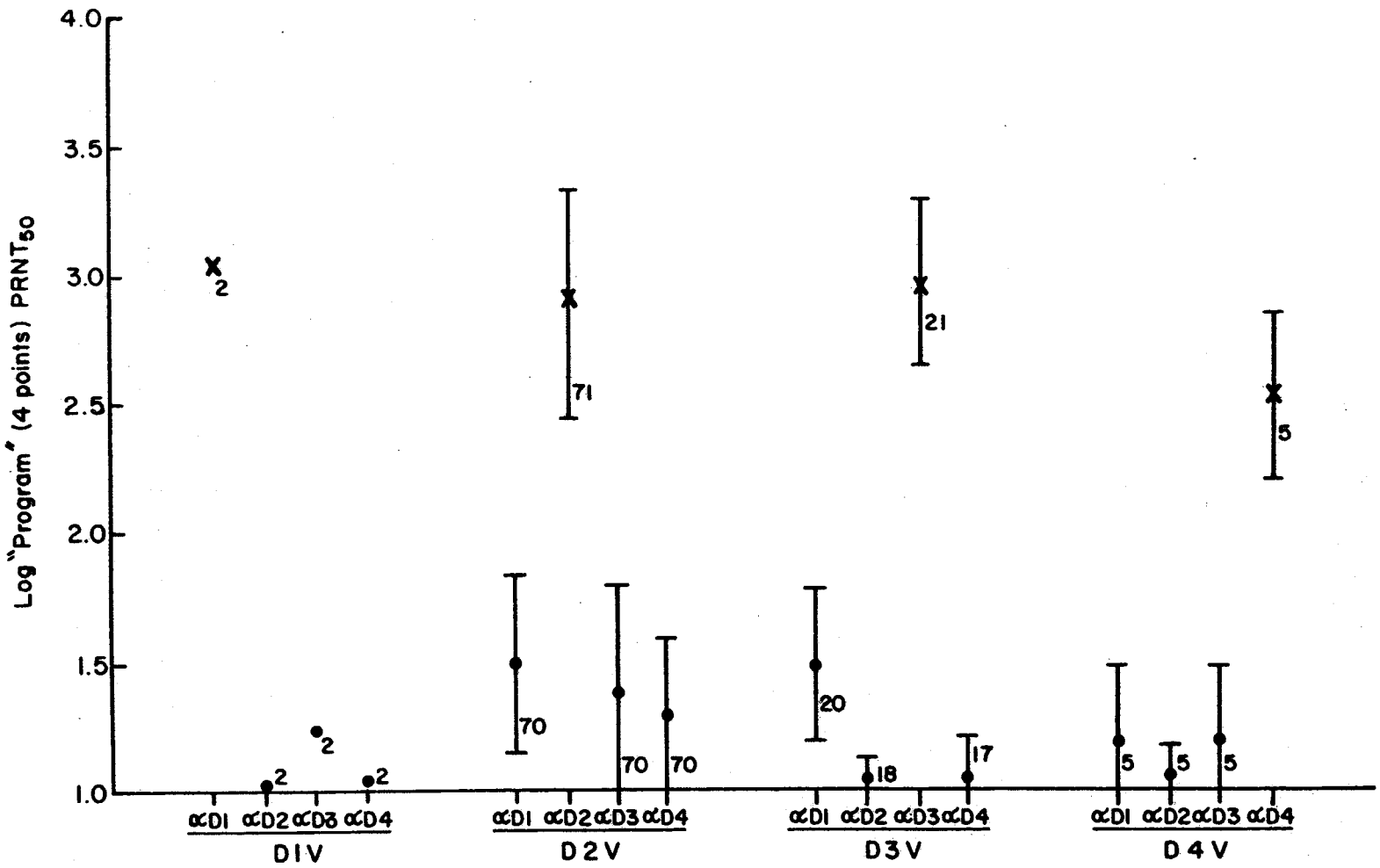
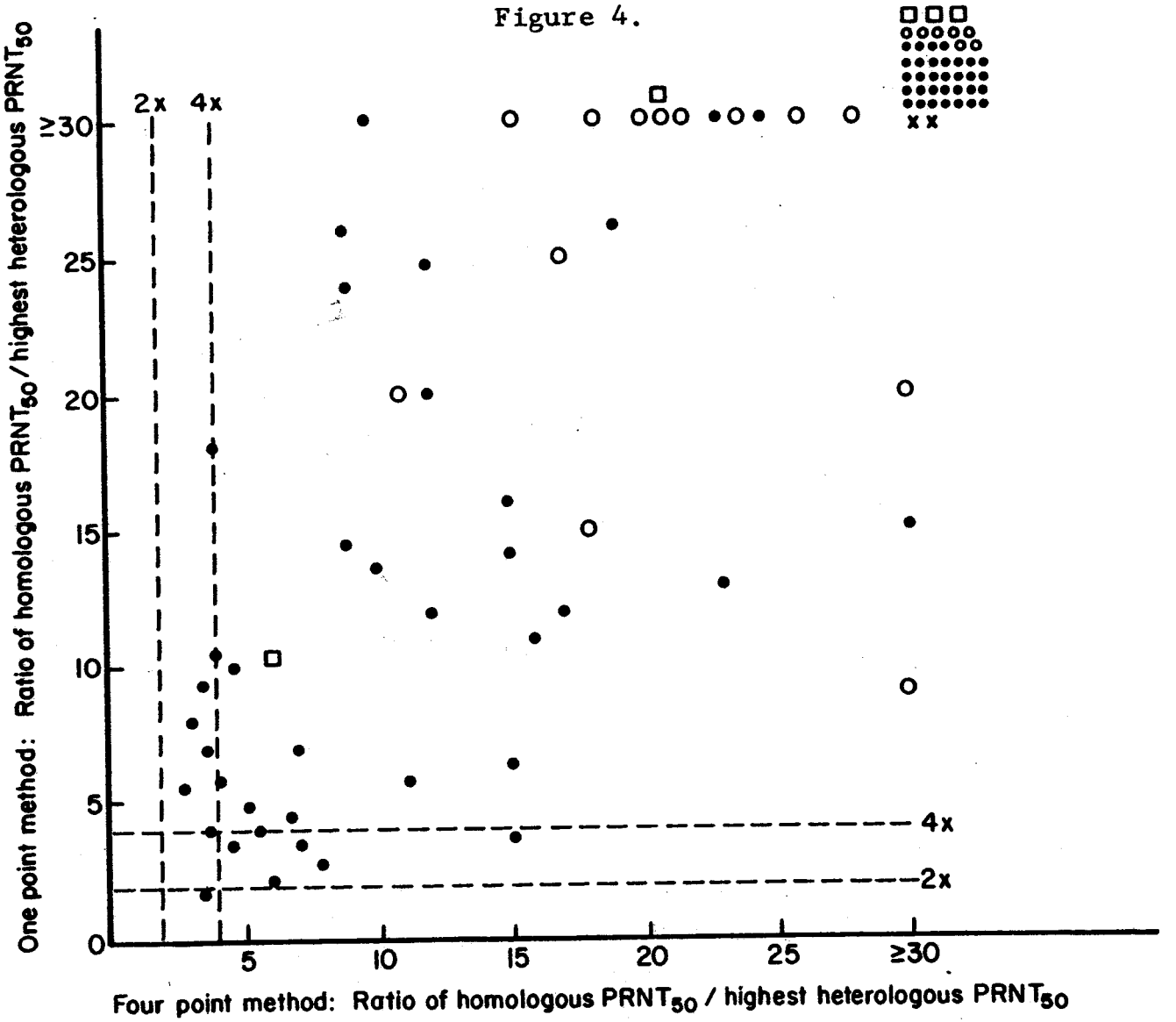

Log "Program" (4 points) PRNT50
4.0
3.5
3.0
2.5
2.0
1.5
1.0
2
2
2
2
71
70
70
70
21
20
18
17
5
5
5
5
αD1 αD2 αD3 αD4
D1V
αD1 αD2 αD3 αD4
D2V
αD1 αD2 αD3 αD4
D3V
αD1 αD2 αD3 αD4
D4V

Figure 4.


One point method: Ratio of homologous PRNT50 / highest heterologous PRNT50
Four point method: Ratio of homologous PRNT50 / highest heterologous PRNT50
2x
4x
4x
2x
0
5
10
15
20
25
≥30