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Two Simple Programs for the Analysis of Data from Enzyme-Linked Immunosorbent (ELISA) Assays on a Programmable Desk-Top Calculator

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We have designed two programs for use with an inexpensive programmable calculator which rapidly and accurately convert raw data generated from enzyme-linked immunosorbent assays directly into antigen concentration. The first program computes and compares effective doses (ED_{50})'s between a standard and each unknown sample assayed. The ED_{50} from the unknown sample is then multiplied by a concentration factor which yields the unknown concentration. The second program linearizes the sigmoidal enzyme-linked immunosorbent assay titration curve using a logit-log transformation of the data in order to compute unknown concentration values. Both programs employ stringent limit conditions to decrease "nonsense" calculations. Data are then processed by a least-squares best-fit linear regression analysis.

Enzyme-linked immunosorbent assays (ELISA)[1] are now used routinely for the quantitative determination of a wide variety of antibodies and soluble antigens (1,2). The sensitivity of this immunoassay is dependent upon the affinity of the antibody to its specific antigen and, under the best of conditions, can rival that of the better known radioimmunoassay (3). ELISAs are usually performed in a 96-well microtiter plate to which either the antigen or antibody has been attached. Basically, the assay is begun by binding a monospecific antibody to the microtiter plate, then exposing the bound antibody to serial dilutions of a solution containing an unknown concentration of the antigen to which the bound antibody is directed. The microtiter plate containing the antigen–antibody mixture is incubated and then washed thoroughly to remove excess unbound antigen. At this point either an enzyme–antibody or an enzyme–antigen label is added. After incubation the bound conjugate is quantitated by the addition of an appropriate substrate which yields a chromogenic product. The amount of chromogen produced is either directly or inversely (depending on the type of conjugate used) related to the amount of antigen bound to the specific antibody. The concentration of the unknown is determined by a graphic transformation of the absorbance readings, and then this curve is compared to that derived from absorbances of known concentrations of the antigen treated in an identical way.

The widespread use and acceptance of this procedure is attested to by the recent development and marketing of spectrophotometers for measuring absorbances directly from microtiter plates. These instruments can easily be connected to low-cost programmable calculators. Although the programs described in this paper were

[1] Abbreviations used: ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; MAS, minimum acceptable slope.

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written specifically for the Texas Instruments TI-59 (cost $200), similar programs could be written for other low-cost programmable calculators that have equivalent data handling and storage capacities. We have designed two simple programs for use with an ELISA plate spectrophotometer directly interfaced with a TI-59 calculator, which allow the conversion of absorbance measurements (from either direct or indirect enzyme immunoassays) into antigen concentrations. Each program processes the absorbance as it is relayed into the calculator. Once the necessary constants obtained from a standard curve are properly stored in the calculator using either program, unknown antigen concentrations can be obtained in a matter of minutes. These constants dictate some of the stringent limit conditions by which the data are subsequently accepted or rejected for linear-regression analysis. These limited conditions decrease the chances of obtaining erroneous results and thereby reduce the necessity of manually graphing each data point.

MATERIALS AND METHODS

Monospecific goat anti-rat fibrinogen, antibody-coated microtiter plates, and fibrinogen–alkaline phosphatase conjugate were prepared as previously described by Kwan *et al.* (4).

Immunosorbent assay. The immunoassays were performed in 96-well round-bottom microtiter plates (Dynatech Laboratories, Inc.) coated with monospecific goat anti-rat fibrinogen (Kwan *et al.* (4)). Briefly, 0.2 ml (5 μg/ml) of rat fibrinogen ($E^{1\%}_{1\,cm}$ = 15.9; λ_{max} 280 nm (5)) in PBS–Tween (0.85% NaCl, 0.05 M phosphate, 0.05% Tween 20, pH 7.1) were placed in row A columns 1 and 2. Samples containing fibrinogen (2–14 μg/ml) were then added (0.2 ml/well) in duplicate or triplicate to row A columns 3–12. Two plates were required for 10 samples plus two standards. Serial twofold dilutions in Tween–saline (0.85% NaCl, 0.05% Tween 20) were made to rows B through G. Row H contained only PBS–Tween throughout the course of the assay. The plates were tightly sealed with cellophane tape and shaken for 2.5 h at 25°C. Unbound antigen was then removed by washing with Tween–saline and antigen–enzyme conjugate (fibrinogen–alkaline phosphatase) was added. The plates were again incubated for 2.5 h, washed with Tween–saline, then incubated with substrate (*p*-nitrophenyl phosphate). The enzymatic reaction was terminated after 15 min by the addition of 0.025 ml of 2 N NaOH. The absorbance of the yellow product, *p*-nitrophenol, was measured at 405 nm with an ELISA plate spectrophotometer (Dynatech).

Calculator-CompuPrint system. The spectrophotometer is interfaced with a CompuPrint 700 (Artek). A Texas Instruments TI-59 programmable calculator, mounted on a PC-100C printer and connected to the CompuPrint, completed the system.

RESULTS

Program I Analysis

When serial twofold dilutions of a 5 μg/ml fibrinogen solution were assayed by the enzyme-linked immunoassay method, a sigmoidal curve relating absorbance to log dilution (i.e., actual concentration) could be drawn through the data (Fig. 1). The general form of the logistic equation which can be used as a model for this relationship may be expressed as

$$Y = \frac{a - d}{1 + (X/c)^b} + d, \qquad [1]$$

where Y is the response; X, the arithmetic concentration, a, the response when $X = 0$; d, the response for "infinite" concentration; c, the ED_{50}, i.e., the concentration resulting from a response halfway between a and d; and b, a "slope factor" that determines the steepness of the curve (6,7). This "slope factor" corresponds to the slope of a logit-log plot (see Fig. 2). It should be emphasized

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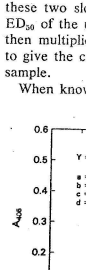

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that the absolute concentration of a sample changes the placement of the curve (i.e., to the right or left) but not its shape. Thus, two or more curves may be characterized separately when compared in terms of slopes and ED_{50}'s. Program I takes advantage of this fact in the following ways: after reading the absorbances from a known fibrinogen standard, the user determines from the program print-out the median absorbance (c), minimum acceptable slope (MAS), and concentration factor (see Appendix I, step 4.1), then enters these values into the appropriate program storage registers. When reading an unknown, slopes between successive dilutions are calculated automatically, then compared with the slope (MAS) obtained for the standard. Once these two slopes match one another, the ED_{50} of the unknown curve is calculated, then multiplied by a concentration factor to give the concentration of the unknown sample.

When known fibrinogen standards were

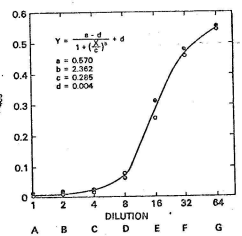


FIG. 1. Standard curve for fibrinogen determination. Plates were coated with 6 μg/ml of monospecific goat anti-rat fibrinogen. Serial twofold dilutions were made (using PBS–Tween) with a rat fibrinogen standard (5.0 μg/ml) from row A through row G. Experimental data (●); theoretical data obtained from Eq. [1] (○); b corresponds to the slope of the logit-log plot (Fig. 2).

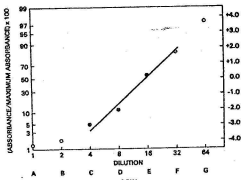


FIG. 2. Logit transformation of absorbances from Fig. 1. Data points from rows A, B, and G (○) were omitted from the linear-regression analysis since they were either less than 3% or greater than 97% of the maximum absorbance (see Discussion). The line describing the binding of enzyme–antigen conjugate was obtained by linear-regression analysis of the four remaining points (●). The correlation coefficient for this line is 0.9906.

assayed and then analyzed with this program, a linear relationship ($r = 0.9957$) between actual versus assayed values was obtained (Fig. 3A). The slope of the linear regression curve was 1.17. Within this range of fibrinogen concentrations the maximum slopes obtained from all samples differed from that of the known calibration standard by 3.4–32.5% (15.5 ± 9.3%, mean ± standard deviation; $N = 10$).

The accuracy of this method is not diminished when the maximum slope obtained from an unknown sample differs by large values from that obtained from a calibration standard. This is attested to by the absolute errors derived from duplicate samples having slopes which are different from a known calibration standard by 3.4 versus 32.5%. These duplicate samples had absolute errors of 1.03 and 0.48 μg/ml, respectively. However, since the majority of unknown samples have maximum slopes which are within ±20% of the calibration standards, we routinely eliminate from analysis all unknown slopes which are less than 80% of that obtained from the standard. This is accomplished

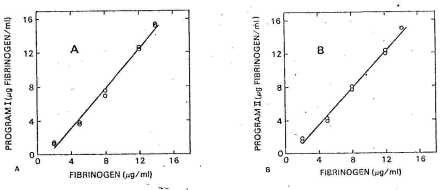


FIG. 3. Comparison of standard curves with known fibrinogen standards using Programs I and II. Using $E^{1\%}_{1cm} = 15.9$ for rat fibrinogen, standards containing between 2 and 14 μg/ml were assayed in duplicate. The resultant absorbances were then converted to fibrinogen concentration (relative to a 5 μg/ml standard) using either Program I (A) or Program II (B). A best-fit linear-regression line has been drawn through each set of data.

automatically with Program I by selecting the appropriate value for a minimum acceptable slope (MAS: see Appendix I, step 4.4). The average absolute error obtained from the fibrinogen values (2 to 14 μg/ml) depicted in Fig. 3A was 0.85 μg/ml. The average absolute error is defined here as the sum of the deviations of the experimental values from the actual values divided by the number of determinations.

Program II Analysis

Program II was written so that the logistic model could be utilized in estimating the concentrations of unknowns. Absorbance measurements were transformed into logit units and then plotted as logit versus log dilution. In this plot a straight line can be drawn through data which the logistic model fits. The logit transformation is given in Eq. [2]:

$$\text{Logit}\ (Y) = \ln\left[\frac{Y}{100 - Y}\right], \quad [2]$$

where Y is the percent response. In this equation Y is defined as

$$Y = (100)\left[\frac{\text{OD}_i}{\text{OD}_{\max}}\right],$$

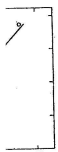
where OD_i is the sample absorbance at dilution i and $\text{OD}_{\max}$ is the absorbance at infinite antigen dilution. Equation [2] may be rewritten as a function of OD_i as follows:

$$f(\text{OD}_i) = \ln\left[\frac{\text{OD}_i}{\text{OD}_{\max} - \text{OD}_i}\right]. \quad [3]$$

ELISA Program II was designed to obtain a linear regression best-fit analysis from the relationship

$$f(\text{OD}_i) = (b)\ \ln\ (\text{dilution}_i) + a, \quad [4]$$

where a is the ordinate intercept and b is the slope. These constants are determined for each regression analysis. A typical logit versus log plot of the data from Fig. 1 is shown in Fig. 2. When the slope, b, from Fig. 2 (obtained from the program print-out for each sample) was entered into Eq. [1], a theoretical sigmoidal curve was obtained which closely approximated the curve obtained from the assay (see Fig. 1). To obtain the slope, the program accepts and averages from one to three absorbance measurements from each row, eliminates values outside of the 3–97% $\text{OD}_{\max}$ range, then converts the remaining values into logit units. The dilutions of known and unknown samples at which the logit equal to zero (as calculated by a least-square linear regression subpro-



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When the same set of known fibrinogen standards were analyzed by this program, a linear relationship ($r = 0.9977$; slope = 1.11) between actual versus experimentally determined values was obtained (Fig. 3B). The average absolute error obtained from Fig. 3B by this method of analysis for rat fibrinogen was 0.46 μg fibrinogen/ml.

DISCUSSION

We have described two simple computer programs each of which is capable of simultaneously analyzing data from enzyme immunoassays while their absorbances are being measured. While both programs are fully automatic when connected to the spectrophotometer-calculator system described in this report, each can be used manually with absorbances being entered through the keyboard followed by keying R/S. Thus, data obtained from any type of spectrophotometer can be rapidly and accurately analyzed using either of these programs. When connected to a spectrophotometer ELISA plate reader, however, the elimination of all manual data manipulations results in a substantial savings in time, as well as preventing operator errors.

Program I utilizes only two points to determine a concentration value. Because the program will automatically extrapolate the line between any two points to the median absorbance (keyed in by the user), erroneous dilution values and consequently erroneous concentration values may be generated. Program I, however, allows the operator to choose an appropriate minimum slope (also obtained from the standard and keyed in by the operator) against which each slope from the unknown is compared. The program then automatically searches the unknown curve for a matching slope before calculating the correct unknown concentration. While the overall accuracy of this program is less than that obtained from the logit analysis performed by Program II, the operator need only measure as many absorbances as necessary to reach an acceptable slope. At this point the concentration is immediately obtained and the next sample can then be analyzed. Thus, plates containing samples having relatively low antigen concentrations may be read in as little as 5–7 min. For this reason, this program is particularly well designed for large-scale screening studies.

Program II calculates antigen concentration by converting absorbances into logit units which are then automatically analyzed by a least-squares linear regression subprogram. One feature of this program is that absorbances outside the range of 3–97% of the maximum observed absorbance are automatically omitted from further analysis. The elimination of these data prevent minor differences in blank absorbances from profoundly affecting the outcome. Generally, five to six points are used for the least-squares best-fit curve. This more detailed method of data analysis results in an increase in accuracy when compared to Program I. Another feature of this program is that the correlation coefficient and slope of each line from the logit plot (for both the standard and all unknowns) are printed. This information allows the operator to reject a calculated sample concentration if discrepancies exist between the unknown in question and the standard. For example, if a correlation coefficient less than 0.95 is obtained for a given sample, the sample should be reassayed. Alternatively, the data may be manually graphed using logit-log paper. If, by visual inspection, a data point was found to deviate significantly from those falling within the 3–97% range, the remaining data points could be manually keyed into the program. Such a procedure would raise the correlation coefficient and improve the accuracy of the calculated antigen concentration. However, this manipulation must be done taking Chauvenet's criterion into consideration (8). This type of

absorbance error can result from differences in the optical path caused by the nonuniform thickness of each plastic well bottom. We have found that both flat-bottom as well as round-bottom plates give similar results. When these extraneous absorbances are accepted into the program, a best-fit line with one or more points substantially deviating from the least-squares curve could be obtained. Thus differences in slopes and low correlation coefficients should then alert the operator to reassay the sample. This type of problem is also prone to occur when the unknown antigen concentration substantially deviates from the concentration of the standard.

In summary, we have written two programs for analysis of data from enzyme-linked immunoassays. Each of the programs contain important constraints which are imposed upon the data in order to assure accurate and reliable data conversion from absorbances and dilutions into concentration units. Both programs are written for use on the TI-59 calculator, an inexpensive calculator that is universally available and easy to use. The use of ELISAs is rapidly increasing in popularity and when used with a system for data analysis as described here is a very convenient and easy approach for the determination of antigen concentrations.

APPENDIX I: DETAILED DESCRIPTION OF PROGRAM I OPERATION

In Table 1, the individual steps for this program are listed. The 462 steps are stored on both sides of one magnetic card. Programming and storage of the program on the magnetic card is performed as described by the manufacturer.

Notes

1.1 Partition calculator to 479.59 by pressing 6; 2nd; Op; 17.
2.1 Insert program card side 1 after pressing INV; 2nd; FIX; CLR. Again press CLR and insert card side 2.
2.2 Press RST; R/S to start program.
3.1 Key in sample number, then press PRINT on printer.
4.1 Enter concentration factor, median absorbance, and minimum acceptable slope as follows:
4.2 Median Absorbance: This is equal to one-half the maximum absorbance obtained from the standard curve. Enter this value and press STO 22.
4.3 Concentration factor (C.F.); for standards enter 1 then press STO; 21. For unknown C.F. = (standard)/dilution; where the dilution is obtained from the standard curve. For example, from Fig. 1, the program calculated a dilution of 16.0. Therefore, C.F. = 5 ÷ 16 = 0.313.
4.4 Minimum acceptable slope (MAS): The slope will vary from antigen to antigen. For fibrinogen the slope is 0.4. For most indirect immunoassays 0.1 can be used initially. Press 0.1; STO 23. Slopes greater than 0.1 will then be printed for each dilution of the standard. From these values the maximum slope is obtained. The MAS = 0.8 x max slope. Enter this value for unknown samples and press STO 23.
4.5 These numbers may all be recorded on tape by pressing TRACE key on printer prior to keying in each of the numbers. Release TRACE before proceeding.
5.1 Before reading absorbances press R/S.

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PROGRAM I															
000	25 CLR	080	71 SBR	161	69 DP	242	05	5	323	01	1	404	03	3
001	69 DP	081	85 +	162	00	00	243	03	3	324	00	0	405	03	3
002	00 00	082	25 CLR	163	03	3	244	02	2	325	00	0	406	01	1
003	03 3	083	69 DP	164	05	5	245	04	4	326	00	0	407	03	3
004	06 6	084	00 00	165	03	3	246	03	3	327	95	=	408	04	04
005	01 1	085	03 3	166	02	2	247	00	0	328	42	STO	409	43	RCL
006	03 3	086	05 5	167	04	4	248	00	0	329	08	08	410	11	11
007	03 3	087	03 3	168	03	3	249	02	2	330	99	PRT	411	85	+
008	00 0	088	02 2	169	00	0	250	01	1	331	91	R/S	412	43	RCL
009	03 3	089	04 4	170	00	0	251	69	DP	332	55	÷	413	12	12
010	03 3	090	03 3	171	01	1	252	01	01	333	01	1	414	95	=
011	02 2	091	00 0	172	06	6	253	69	DP	334	00	0	415	55	÷
012	07 7	092	00 0	173	69	DP	254	05	05	335	00	0	416	02	2
013	69 DP	093	01 1	174	01	01	255	71	SBR	336	00	0	417	95	=
014	01 01	094	04 4	175	69	DP	256	33	X²	337	95	=	418	42	STO
015	01 1	095	69 DP	176	05	05	257	25	CLR	338	42	STO	419	13	13
016	07 7	096	01 01	177	71	SBR	258	36	PGM	339	09	09	420	69	DP
017	69 DP	097	69 DP	178	33	X²	259	01	01	340	99	PRT	421	06	06
018	00 0	098	05 05	179	25	CLR	260	71	SBR	341	25	CLR	422	98	ADV
019	00 0	099	71 SBR	180	36	PGM	261	25	CLR	342	69	DP	423	92	RTN
020	00 0	100	33 X²	181	01	01	262	43	RCL	343	00	00	424	76	LBL
021	00 0	101	25 CLR	182	71	SBR	263	18	18	344	03	3	425	11	A
022	00 0	102	36 PGM	183	25	CLR	264	32	XIT	345	00	0	426	69	DP
023	00 0	103	01 01	184	43	RCL	265	43	RCL	346	00	0	427	12	12
024	00 0	104	71 SBR	185	16	16	266	10	10	347	00	0	428	95	=
025	69 DP	105	25 CLR	186	32	XIT	267	78	Σ+	348	01	1	429	32	XIT
026	02 02	106	43 RCL	187	43	RCL	268	43	RCL	349	07	7	430	95	=
027	69 DP	107	14 14	188	10	10	269	19	19	350	01	1	431	42	STO
028	05 05	108	32 XIT	189	78	Σ+	270	32	XIT	351	03	3	432	24	24
029	71 SBR	109	43 RCL	190	43	RCL	271	43	RCL	352	03	3	433	69	DP
030	33 X²	110	17 17	191	17	17	272	13	13	353	01	1	434	43	RCL
031	91 R/S	111	78 Σ+	192	32	XIT	273	78	Σ+	354	00	0	435	23	23
032	25 CLR	112	43 RCL	193	43	RCL	274	43	RCL	355	04	04	436	32	XIT
033	69 DP	113	15 15	194	13	13	275	22	22	356	43	RCL	437	25	CLR
034	00 00	114	32 XIT	195	78	Σ+	276	11	A	357	08	08	438	43	RCL
035	03 3	115	43 RCL	196	43	RCL	277	25	CLR	358	85	+	439	24	24
036	05 5	116	13 13	197	22	22	278	69	DP	359	43	RCL	440	95	=
037	05 5	117	78 Σ+	198	11	A	279	00	00	360	09	09	441	77	GE
038	02 2	118	43 RCL	199	25	CLR	280	03	3	361	55	÷	442	04	04
039	04 4	119	22 22	200	69	DP	281	05	5	362	55	÷	443	47	47
040	03 3	120	25 CLR	201	00	00	282	03	3	363	02	2	444	92	RTN
041	00 0	121	69 DP	202	03	3	283	02	2	364	95	=	445	43	RCL
042	01 1	122	00 00	203	05	5	284	04	4	365	42	STO	446	24	24
043	69 DP	123	03 3	204	03	3	285	03	3	366	10	10	447	95	=
044	01 01	124	03 3	205	02	2	286	00	0	367	09	09	448	98	PRT
045	69 DP	125	05 5	206	04	4	287	00	0	368	06	06	449	43	RCL
046	05 05	126	02 2	207	03	3	288	00	0	369	98	ADV	450	22	22
047	01 1	127	02 2	208	00	0	289	02	2	370	92	RTN	451	69	DP
048	28 LOG	128	04 4	209	00	0	290	69	DP	371	76	LBL	452	15	15
049	42 STO	129	03 3	210	01	1	291	01	01	372	33	X²	453	95	=
050	14 14	130	00 0	211	07	7	292	69	DP	373	25	CLR	454	32	INV
051	02 2	131	01 1	212	69	DP	293	05	05	374	69	DP	455	28	LOG
052	28 LOG	132	01 1	213	01	01	294	71	SBR	375	55	÷	456	65	×
053	42 STO	133	05 5	214	69	DP	295	33	X²	376	01	1	457	43	RCL
054	15 15	134	69 DP	215	05	05	296	25	CLR	377	00	0	458	21	21
055	04 4	135	01 01	216	71	SBR	297	36	PGM	378	00	0	459	95	=
056	28 LOG	136	69 DP	217	85	+	298	01	01	379	00	0	460	98	ADV
057	42 STO	137	05 05	218	25	CLR	299	71	SBR	380	95	=	461	98	ADV
058	16 16	138	71 SBR	219	36	PGM	300	25	CLR	381	42	STO	462	92	RTN
059	08 8	139	85 +	220	01	01	301	43	RCL	382	11	11			
060	28 LOG	140	25 CLR	221	71	SBR	302	19	19	383	99	PRT			
061	42 STO	141	36 PGM	222	25	CLR	303	32	XIT	384	91	R/S			
062	17 17	142	01 01	223	43	RCL	304	43	RCL	385	55	÷			
063	01 1	143	71 SBR	224	17	17	305	13	13	386	01	1			
064	06 6	144	25 CLR	225	32	XIT	306	78	Σ+	387	00	0			
065	28 LOG	145	43 RCL	226	43	RCL	307	43	RCL	388	00	0			
066	42 STO	146	15 15	227	13	13	308	20	20	389	00	0			
067	18 18	147	32 XIT	228	78	Σ+	309	32	XIT	390	95	=			
068	03 3	148	43 RCL	229	43	RCL	310	43	RCL	391	42	STO			
069	02 2	149	13 13	230	18	18	311	10	10	392	12	12			
070	28 LOG	150	78 Σ+	231	32	XIT	312	78	Σ+	393	99	PRT			
071	42 STO	151	43 RCL	232	43	RCL	313	43	RCL	394	25	CLR			
072	19 19	152	16 16	233	10	10	314	22	22	395	98	ADV			
073	06 6	153	32 XIT	234	78	Σ+	315	11	A	396	00	00			
074	02 2	154	43 RCL	235	43	RCL	316	25	CLR	397	03	3			
075	28 LOG	155	10 10	236	22	22	317	81	RST	398	00	0			
076	42 STO	156	78 Σ+	237	11	A	318	76	LBL	399	03	3			
077	20 20	157	43 RCL	238	25	CLR	319	85	+	400	00	0			
078	58 FIX	158	22 22	239	69	DP	320	25	CLR	401	00	0			
079	03 03	159	11 A	240	00	00	321	91	R/S	402	07	7			
		160	25 CLR	241	03	3	322	55	÷	403	01	1			

6.1 Read Absorbance from standard row A column 1. When using a MicroELISA Reader press PRINT button on Reader. Absorbances will then be automatically entered into the program. When using a spectrophotometer that is not interfaced to a calculator, absorbances must be manually keyed in as a whole number followed R/S.

6.2 Read row A column 2.

6.3 Read row B columns 1 and 2. Be careful not to enter new data unless a

0 appears in calculator display. If new data are accidentally entered while calculations are in progress, the program overloads and must be rerun from the beginning by pressing RST; R/S; R/S and beginning again at row A column 1.

6.4 Continue reading each successive row. Print-out will include mean absorbance for each row followed by two numbers if the slope is greater than MAS in register 23. The first number is the slope while the second is either the dilution (when reading the standard) or the final antigen concentration (when reading an unknown).

7.1 To begin reading another sample press RST; R/S. Be sure storage register 21, 22, and 23 contain the appropriate numbers for either standards or unknowns.

APPENDIX II: DETAILED DESCRIPTION OF PROGRAM II OPERATION

In Table 2, the individual steps for Program II are listed. The 612 program steps are stored on three sides of two magnetic cards. Programming and storage of the program are performed as described by the manufacturer.

Notes

1.1 Partition calculator to 719.29. Press 3; 2nd; Op; 17.

1.2 Enter the program stored on magnetic cards. Press RST; CLR. Insert card side 1. Press CLR, insert card side 2. Press CLR, insert card side 3.

2.1 Scan the ELISA plate for the highest single absorbance measurement. Multiply that reading by 1000, and store it in register 21. Example: if the highest absorbance measurement is 0.632, enter 632 and press STO 21. Press RST, CLR.

3.1 To start the program press R/S, R/S.

3.2 The calculator will then ask "CONC OF STD IS?" Enter from the keyboard the undiluted concentration of the standard solution of antigen. Press R/S.

3.3 The calculator will then print "1 = STD 0 = UNK." Press 1, R/S, if the set of data about to be entered is a *stand*ard. Press 0, R/S, if the set of data to be entered is a solution of *unk*nown antigen concentration.

3.4 The calculator will then print "$\bar{X}$ OF 1, 2, OR 3 ODS?" Press 1, R/S, if the data to be entered are singlets. Press 2, R/S, if the data are in duplicate. Press 3, R/S, if the data are in triplicate.

3.5 The calculator will then print "OD IS." Make sure that the microtiter plate is positioned at the first well to be measured (position A-1), then press the PRINT button on the ELISA spectrophotometer. The absorbance is automatically entered into the calculator. If the calculator and spectrophotometer are not interfaced, multiply the absorbance by 1000, enter that number and press R/S. The calculator will then print "OD IS." Enter the next measurement. If samples are assayed as duplicates read position A-2. The calculator will print the mean of the two absorbance measurements. If data are in triplicates read well A-3 and the calculator will print the mean of the three absorbances.

3.6 Be sure to wait until the calculator has finished all calculations before entering data. When "C" appears in the calculator display left-hand corner, the

PROGRAM II		
000	91	R/S
001	25	CLR
002	69	DP
003	00	00
004	01	1
005	05	5
006	00	0
007	01	1
008	03	3
009	01	1
010	01	1
011	05	5
012	00	0
013	00	0
014	69	DP
015	01	01
016	00	0
017	01	1
018	02	2
019	01	1
020	00	0
021	00	0
022	03	3
023	06	6
024	03	3
025	07	7
026	69	DP
027	02	02
028	91	1
029	04	4
030	00	0
031	00	0
032	02	2
033	04	4
034	03	3
035	06	6
036	07	7
037	01	1
038	69	DP
039	03	03
040	69	DP
041	05	05
042	91	R/S
043	42	STO
044	10	10
045	99	PRT
046	76	LBL
047	15	E
048	00	0
049	42	STO
050	01	01
051	42	STO
052	02	02
053	42	STO
054	03	03
055	42	STO
056	04	04
057	42	STO
058	05	05
059	42	STO
060	06	06
061	42	STO
062	07	07
063	42	STO
064	00	00
065	42	STO
066	09	09
067	42	STO
068	11	11
069	42	STO
070	15	15
071	42	STO
072	17	17
073	42	STO
074	18	18
075	42	STO
076	20	20
077	42	STO
078	19	19
079	42	STO

TABLE 2

PROGRAM II			
000	91	R/S	
001	25	CLR	
002	69	DP	
003	00	00	
004	01	1	
005	05	5	
006	00	0	
007	01	1	
008	03	3	
009	01	1	
010	00	0	
011	05	5	
012	00	0	
013	00	0	
014	69	DP	
015	01	01	
016	00	0	
017	01	1	
018	02	2	
019	01	1	
020	00	0	
021	00	0	
022	03	3	
023	00	0	
024	03	3	
025	00	0	
026	69	DP	
027	02	02	
028	01	1	
029	06	6	
030	00	0	
031	00	0	
032	02	2	
033	04	4	
034	03	3	
035	06	6	
036	07	7	
037	01	1	
038	69	DP	
039	03	03	
040	69	DP	
041	05	05	
042	91	R/S	
043	42	STO	
044	10	10	
045	99	PRT	
046	76	LBL	
047	15	E	
048	00	0	
049	42	STO	
050	01	01	
051	42	STO	
052	02	02	
053	42	STO	
054	03	03	
055	42	STO	
056	04	04	
057	42	STO	
058	05	05	
059	42	STO	
060	06	06	
061	42	STO	
062	07	07	
063	42	STO	
064	08	08	
065	42	STO	
066	09	09	
067	42	STO	
068	11	11	
069	42	STO	
070	15	15	
071	42	STO	
072	17	17	
073	42	STO	
074	18	18	
075	42	STO	
076	20	20	
077	42	STO	
078	19	19	
079	42	STO	
080	22	22	
081	42	STO	
082	24	24	
083	25	CLR	
084	69	DP	
085	00	00	
086	03	0	
087	00	0	
088	06	6	
089	04	4	
090	03	3	
091	06	6	
092	03	3	
093	07	7	
094	01	1	
095	06	6	
096	69	DP	
097	01	01	
098	01	1	
099	01	1	
100	06	6	
101	04	4	
102	04	4	
103	01	1	
104	03	3	
105	01	1	
106	07	7	
107	06	6	
108	69	DP	
109	02	02	
110	49	DP	
111	05	05	
112	91	R/S	
113	42	STO	
114	12	12	
115	25	CLR	
116	00	00	
117	69	DP	
118	00	00	
119	06	6	
120	07	7	
121	00	0	
122	00	0	
123	00	0	
124	04	4	
125	07	7	
126	06	6	
127	00	0	
128	00	0	
129	69	DP	
130	04	04	
131	00	0	
132	02	2	
133	05	5	
134	07	7	
135	00	0	
136	03	3	
137	05	5	
138	07	7	
139	00	0	
140	69	DP	
141	05	05	
142	02	02	
143	01	1	
144	01	1	
145	05	5	
146	05	5	
147	00	0	
148	00	0	
149	00	0	
150	04	4	
151	00	0	
152	00	0	
153	69	DP	
154	03	03	
155	01	1	
156	05	5	
157	06	6	
158	05	5	
159	06	6	
160	07	7	
161	01	1	
162	69	DP	
163	04	04	
164	69	DP	
165	05	05	
166	91	R/S	
167	99	PRT	
168	42	STO	
169	17	17	
170	42	STO	
171	08	08	
172	08	8	
173	42	STO	
174	09	09	
175	00	0	
176	42	STO	
177	07	07	
178	61	GTO	
179	01	01	
180	92	92	
181	76	LBL	
182	12	B	
183	00	0	
184	42	STO	
185	22	22	
186	43	RCL	
187	17	17	
188	42	STO	
189	00	00	
190	76	LBL	
191	11	A	
192	76	LBL	
193	13	C	
194	69	DP	
195	25	CLR	
196	69	DP	
197	00	00	
198	01	1	
199	01	1	
200	00	0	
201	06	6	
202	00	0	
203	00	0	
204	02	2	
205	04	4	
206	03	3	
207	06	6	
208	69	DP	
209	00	00	
210	69	DP	
211	01	01	
212	91	R/S	
213	99	PRT	
214	44	SUM	
215	22	22	
216	69	DP	
217	02	02	
218	08	08	
219	01	01	
220	90	90	
221	25	CLR	
222	69	DP	
223	00	00	
224	03	3	
225	00	0	
226	01	1	
227	07	7	
228	00	0	
229	03	3	
230	01	1	
231	01	1	
232	00	0	
233	04	04	
234	43	RCL	
235	22	22	
236	69	DP	
237	43	RCL	
238	55	÷	
239	19	19	
240	69	DP	
241	06	06	
242	42	STO	
243	20	20	
244	55	÷	
245	53	(	
246	43	RCL	
247	21	21	
248	75	-	
249	43	RCL	
250	20	20	
251	54	)	
252	95	=	
253	23	LNX	
254	42	STO	
255	24	24	
256	43	RCL	
257	20	20	
258	55	÷	
259	43	RCL	
260	21	21	
261	95	=	
262	42	STO	
263	19	19	
264	02	2	
265	45	Y^x	
266	43	RCL	
267	07	07	
268	95	=	
269	42	STO	
270	18	18	
271	23	LNX	
272	42	STO	
273	11	11	
274	69	DP	
275	27	27	
276	1	1	
277	00	0	
278	03	3	
279	32	X↕T	
280	43	RCL	
281	19	19	
282	22	INV	
283	77	GE	
284	03	03	
285	04	04	
286	93	.	
287	09	9	
288	07	7	
289	32	X↕T	
290	45	RCL	
291	19	19	
292	77	GE	
293	03	03	
294	04	04	
295	43	RCL	
296	11	11	
297	32	X↕T	
298	43	RCL	
299	24	24	
300	78	Σ+	
301	61	GTO	
302	03	03	
303	23	23	
304	76	LBL	
305	18	C'	
306	25	CLR	
307	69	DP	
308	00	00	
309	00	0	
310	01	1	
311	09	9	
312	00	0	
313	02	2	
314	04	4	
315	03	3	
316	07	7	
317	69	DP	
318	04	04	
319	43	RCL	
320	19	19	
321	69	DP	
322	06	06	
323	76	LBL	
324	19	D'	
325	07	7	
326	32	X↕T	
327	43	RCL	
328	07	07	
329	67	EQ	
330	03	03	
331	00	00	
332	61	GTO	
333	01	01	
334	81	81	
335	76	LBL	
336	17	B'	
337	00	0	
338	69	DP	
339	15	15	
340	22	INV	
341	23	LNX	
342	42	STO	
343	13	13	
344	25	CLR	
345	69	DP	
346	00	00	
347	01	1	
348	06	6	
349	02	2	
350	04	4	
351	02	2	
352	07	7	
353	03	3	
354	01	1	
355	00	0	
356	00	0	
357	69	DP	
358	01	01	
359	01	1	
360	03	3	
361	03	3	
362	07	7	
363	00	0	
364	00	0	
365	02	2	
366	07	7	
367	00	0	
368	01	1	
369	69	DP	
370	02	02	
371	02	2	
372	02	2	
373	02	2	
374	04	4	
375	03	3	
376	07	7	
377	06	6	
378	04	4	
379	00	0	
380	01	1	
381	69	DP	
382	03	03	
383	69	DP	
384	05	05	
385	43	RCL	
386	13	13	
387	99	PRT	
388	25	CLR	
389	69	DP	
390	00	00	
391	02	2	
392	06	6	
393	02	2	
394	07	7	
395	00	0	
396	01	1	
397	03	3	
398	03	3	
399	01	1	
400	07	7	
401	69	DP	
402	01	01	
403	69	DP	
404	05	05	
405	69	DP	
406	12	12	
407	32	X↕T	
408	99	PRT	
409	25	CLR	
410	69	DP	
411	00	00	
412	01	1	
413	05	5	
414	00	0	
415	01	1	
416	01	1	
417	05	5	
418	03	3	
419	05	5	
420	00	0	
421	00	0	
422	69	DP	
423	01	01	
424	01	1	
425	05	5	
426	00	0	
427	01	1	
428	01	1	
429	07	7	
430	02	2	
431	01	1	
432	02	2	
433	01	1	
434	69	DP	
435	02	02	
436	49	DP	
437	05	05	
438	69	DP	
439	13	13	
440	99	PRT	
441	01	1	
442	05	5	
443	43	RCL	
444	12	12	
445	67	EQ	
446	05	05	
447	96	96	
448	25	CLR	
449	69	DP	
450	00	00	
451	02	2	
452	01	1	
453	03	3	
454	01	1	
455	02	2	
456	06	6	
457	04	4	
458	00	0	
459	01	1	
460	05	5	
461	69	DP	
462	01	01	
463	00	0	
464	01	1	
465	03	3	
466	01	1	
467	01	1	
468	04	4	
469	06	6	
470	04	4	
471	00	0	
472	01	1	
473	69	DP	
474	02	02	
475	69	DP	
476	05	05	
477	43	RCL	
478	10	10	
479	65	×	
480	43	RCL	
481	13	13	
482	55	÷	
483	43	RCL	
484	14	14	
485	95	=	
486	99	PRT	
487	25	CLR	
488	69	DP	
489	00	0	
490	01	1	
491	06	6	
492	00	0	
493	01	1	
494	03	3	
495	05	5	
496	01	1	
497	07	7	
498	07	7	
499	01	1	
500	69	DP	
501	01	01	
502	00	0	
503	02	2	
504	06	6	
505	04	4	
506	04	4	
507	05	5	
508	01	1	
509	07	7	
510	03	3	
511	01	1	
512	69	DP	
513	02	02	
514	00	0	
515	00	0	
516	00	0	
517	01	1	
518	06	6	
519	00	0	
520	03	3	
521	00	0	
522	03	3	
523	01	1	
524	69	DP	
525	03	03	
526	69	DP	
527	05	05	
528	43	RCL	
529	13	13	
530	15	15	
531	99	PRT	
532	25	CLR	
533	69	DP	
534	00	00	
535	01	1	
536	07	7	
537	00	0	
538	04	4	
539	25	CLR	
540	69	DP	
541	00	00	
542	01	1	
543	05	5	
544	01	1	
545	07	7	
546	01	1	
547	07	7	
548	01	1	
549	03	3	
550	02	2	
551	01	1	
552	69	DP	
553	01	01	
554	02	2	
555	00	0	
556	01	1	
557	03	3	
558	05	5	
559	05	5	
560	01	1	
561	06	6	
562	01	1	
563	03	3	
564	69	DP	
565	02	02	

566	00	0
567	00	0
568	03	3
569	01	1
570	02	2
571	01	1
572	01	1
573	03	3
574	03	3
575	01	1
576	69	DP
577	03	03
578	69	DP
579	06	6
580	01	1
581	06	6
582	07	7
583	03	3
584	07	7
585	03	3
586	00	0
587	00	0
588	69	DP
589	04	04
590	49	DP
591	05	05
592	61	GTO
593	06	06
594	47	47
595	76	LBL
596	17	B'
597	43	RCL
598	13	13
599	42	STO
600	14	14
601	00	0
602	45	Y^x
603	13	13
604	61	GTO
605	00	00
606	47	47
607	76	LBL
608	17	B'
609	68	NOP
610	91	R/S
611	81	RST

Insert card side 3.

ient. Multi- the highest) 21. Press

ie keyboard Press R/S. R/S, if the set of ation. ress 1, R/S, are in dupli-

crotiter plate , then press bsorbance is and spectro-)0, enter that ;." Enter the position A-2. easurements. will print the

fore entering d corner, the

calculator is still processing data. Wait until the "C" is no longer displayed before entering more data.

3.7 Proceed to the next dilutions, in order, rows B through G and enter those data pairs, singlets, or triplets to be averaged.

3.8 At the end of each set of dilutions the calculator will perform a least-squares analysis of the data. It will print "DILN AT LOGIT = 0." This phrase means the dilution of the sample, calculated by least-squares analysis, that would

[4], where $f(OD_i) = 0$. The dilution at logit = 0 is equivalent to the ED_{50}. This dilution is used in further calculations to determine the sample concentration. The slope of the least-squares fit and the correlation coefficient are also printed. If the sample just entered was an unknown the calculator will print "UNK CONC =" followed by the *unk*nown *con*centration. The dimensions of the unknown concentration are the same as those of the standard concentration.

3.9 The calculator will print "DONE? 1 = YES 0 = NO." If finished with the program, push 1, R/S. If not finished, press 0, R/S.

4.1 In certain cases, absorbance measurements are omitted from analysis. Data points outside the range of 3–97% of the maximum absorbance are omitted. These data are printed and identified by the calculator. After the calculator prints the mean absorbance, it will print the ratio of the mean absorbance to the maximum absorbance followed by "OMIT."

4.2 Be sure to read all wells in a row. If the ELISA assay makes use of more or less than rows A through G, an adjustment in the program must be made. Change step 325 from 7 to the number of wells used in a single row of dilutions.

4.3 If other than serial twofold dilutions are used in an ELISA assay, Program II may be modified as follows: Change step 264 from 2 to any single integer (3–9 inclusive) which describes the fold dilution.

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A Rapi

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We prese
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It is often ne
DNA content of c
lished procedures
many purificatio
cedure describe
shorter and easie
sors; yet it does
sensitivity. An
technique is that
tein, thus allowi
tein in the same
sensitivity make
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DNA determinat

MATERIA

Highly polym
was purchased fi
Corporation as a
a 1 mg/ml stock
1 N NH_4OH, an
taken to drynes
zoic acid dihydr
was obtained

[1] Abbreviations u
acid; TCA, trichlor