Enzyme-linked Immunosorbent Assay (ELISA) on Japanese Encephalitis Virus

IV. A computer system to calculate ELISA endpoint titer from ELISA-OD at a single dilution of test sera

Kouichi MORITA, Keiko BUNDO, and Akira IGARASHI

Department of Virology, Institute for Tropical Medicine, Nagasaki University

Abstract: A computer program was worked out to calculate ELISA endpoint titers from the ELISA-OD obtained at a single dilution of test sera. The procedure, formerly performed by reading the values from a graphical curve on semilogarithmic scale chart, was very much reduced in time and enabled statistical analysis on the data in epidemiological studies much more efficient and accurate.

INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) has been introduced, since its primary description by Engvall and Perlman (1971), to various fields of infectious diseases, such as virology (WHO, 1979), bacteriology (Pope *et al.*, 1982), and parasitology (Ambroise-Thomas *et al.*, 1981a; b), because of its rapidity, simplicity and capacity to deal with a number of specimens. We have been studying the application of the ELISA to measure antibody titers against Japanese encephalitis (JE) virus in human and swine sera (Igarashi *et al.*, 1981; Bundo *et al.*, 1981; 1982). Several people tried to show the ELISA titer in test sera by the reciprocal of the highest dilution of the sera (end-point titer) showing significant difference between the positive and negative antigen or observed OD value itself (Voller *et al.*, 1976). Other people used the difference between the dose-response curve of the test sera and that of the standard positive serum obtained on a semilogarithmic scale (Leinikki and Passila, 1976). The characteristics of our system is to estimate ELISA endpoint titers of test sera from ELISA-OD obtained at a single (or a few) dilution(s) comparing the OD with the standard curve obtained by serial 2-fold dilution of a standard positive serum of known endpoint titer (Igarashi *et al.*, 1981). This method has advantages of reproducibility and capacity to handle large number of

Received for publication, September 13, 1982.
Contribution No. 1246 from the Institute for Tropical Medicine, Nagasaki University.

specimens, however, required quite a bit of time in reading the "titer" from the ELISA-OD using standard curve drawn on a semilogarithmic scale paper. Recent advance in computer technology is remarkable and made various procedures of calculation or estimation far more efficient than before. We tried to work out a program system using ordinaly "Office Computer" in order to calculate ELISA endpoint titer from ELISA-OD obtained at a single dilution of test sera, comparing the standard curve obtained by serial dilutions of standard serum.

MATERIALS AND METHODS

Computer system: Personal computer (PC-8801; NEC); Disk unit (PC-8881; NEC); Dot matrix printer (PC-8821, NEC); and Monochrome display monitor (PC-8851) were used.

program word: n88-basic was used.

ELISA: Indirect micro ELISA (Voller *et al.*, 1976) was used with modifications as described before (Igarashi *et al.*, 1981).

RESULTS

Fig. 1 shows an example of our computer program worked out for our purpose. Here, we have to put the ELISA-OD values obtained for serial 2-fold dilution of a standard positive serum into No. 1 to No. 10, starting from the higher OD-value. Usually duplicate tests were run for each dilution of the standard serum. Next, sample number and ELISA-OD value for each test specimen is put in. Then, following "calculate", dilution of standard serum, dilution of test specimen, titer of standard serum and cut-off No.* are put in, resulting in the drawing of the standard curve and printing put of the ELISA titer for each specimen (Fig. 2). This procedure enables us to ascertain that the test was run successfully or not by looking at the standard curve. Previous procedures using a graphical method on a semilogarithmic scale paper required more than an hour to read the titer for a single microplate. However, the computer system could perform the same procedure within 15 minutes.

Table 1 compares the ELISA titers obtained for 20 independent serum specimens by the previous graphical method and by the computer method, showing good correlation and confirming the validity of the method. There will be no mistake in the titer as far as the input OD-value and other data are correct.

---

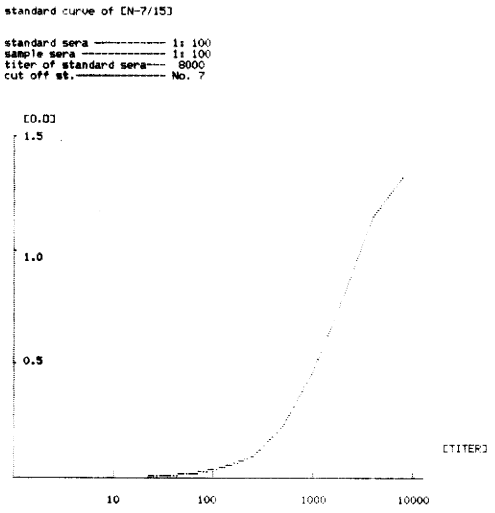
*Cut off No. The specimens showing ELISA-ODs lower than the values by the standard serum at this No. of dilution are cut off and are shown by sign of inequality in printing out.

```
1000 '
1010 '          ELISA program n88-BASIC
1020 '
1030 '                1982/4
1040 '
1050 DIM Y(100):DIM M(100):DIM V(100):DIM Z(20)
1060 DIM W(100):DIM MM(100):DIM N(100)
1070 DIM A(100,5): DIM X(100)
1080 WIDTH 80,25:PRINT CHR$(12)
1090 KEY 1,"y"+CHR$(13):KEY 2,""
1100 KEY 3,"run"+CHR$(13)
1110 KEY 4,"":KEY 5,"no"+CHR$(13)
1120 PRINT :PRINT"      ELISA program"
1130 PRINT :PRINT :PRINT
1140 PRINT "  O.D. input  ----------(1)"
1150 PRINT "  O.D. output ----------(2)"
1160 PRINT "  O.D. correct----------(3)"
1170 PRINT "  caluculate titers-----(4)"
1180 PRINT "  END ----------------------(10)"
1190 INPUT G
1200 IF G=10 THEN  END
1210 INPUT "input plate name ";A$
1220 K=0
1230 INPUT"input code initial of samples";L$
1240 IF G=1 GOTO 1300
1250 IF G=2 GOTO 1580
1260 IF G=3 GOTO 1580
1270 IF G=4 GOTO 2510
1280 GOTO 1080
1290 ' O.D. input
1300 OPEN A$    FOR OUTPUT AS #1
1310 PRINT CHR$(12)
1320 PRINT "number of samples ";
1321 PRINT " (including standards)";
1330 INPUT M
1340 PRINT #1,M
1350 FOR I=1 TO M
1360 IF I<=10 THEN GOTO 1390
1370 IF I>=11 THEN N=1
1380 PRINT :PRINT "data No.";I: GOTO 1430
1390 PRINT"Input tne number of stnd. No.";
1391 PRINT I;" = ";
1400 INPUT N
1410 PRINT
1420 IF I=<10 THEN MM=0 :GOTO 1450
1430 PRINT "sample code";L$;"-";
1440 INPUT MM
1450 FOR J=1 TO N
1460 PRINT "No.";J;"=";
1470 INPUT A(I,J)
1480 PRINT :PRINT
1490 NEXT J
1500 PRINT #1,MM
1510 PRINT #1,N
1520 FOR J=1 TO N
1530 PRINT #1,A(I,J)
1540 NEXT J
1550 NEXT I
1560 CLOSE
1570 'O.D. output
1580 OPEN A$   FOR INPUT AS #1
1590 I=0
1600 INPUT #1,M
1610 IF EOF(1) THEN GOTO 1690
1620 I=I+1
1630 INPUT #1,MM(I)
1640 INPUT #1,N(I)
1650 FOR J=1 TO N(I)
1660 INPUT #1,A(I,J)
1670 NEXT J
1680 GOTO 1610
1690 FOR I=1 TO 10
1700 Q=0
1710 FOR J=1 TO N(I)
1720 Q=Q+A(I,J)
1730 NEXT J
1740 W(I)=Q/N(I)
1750 PRINT "No. ";
1760 PRINT USING "##";I;:PRINT "  ";
1770 PRINT "st.-";:PRINT USING"##";I;
1780 PRINT "   C";
1790 PRINT USING "#.###";W(I);
1800 PRINT "J ";
1810 FOR J=1 TO N(I)
1820 PRINT USING"#.###";A(I,J);:PRINT " ";
1830 NEXT J
1840 PRINT
1850 NEXT I
1860 FOR I=11 TO M
1870 PRINT "No. ";:PRINT USING "##";I;
1880 PRINT "  ";L$;"-";
1890 PRINT USING "###";MM(I);:PRINT "     ";
1900 PRINT USING "#.###";A(I,1)
1910 NEXT I
1920 IF K=3 GOTO 2170
1930 CLOSE
1940 IF G=3 GOTO 2010
1950 INPUT "Do you correct (y) or (no)";E$
1960 IF E$="y" THEN GOTO 2010
1970 INPUT"print ?  (y) or (no)";D$
1980 IF D$="y"THEN GOTO 2300
1990 GOTO 1140
2000 ' correct
2010 K=3:INPUT "How many mistakes";H
2020 FOR I=1 TO H
2030 INPUT "mistake No.";M1
2040 IF M1=<10 GOTO 2090
2050 INPUT "code No.";M2
2060 INPUT "O.D.";M3
2070 A(M1,1)=M3:MM(M1)=M2
2080 GOTO 2140
2090 INPUT "input number of data";M4
2100 N(M1)=M4
2110 FOR J=1 TO M4
2120 INPUT "O.D.";A(M1,J)
2130 NEXT J
2140 NEXT I
2150 G=0
2160 GOTO 1690
2170 INPUT "Is it O.K.? (y) or (no)";Y$
2180 IF Y$="no" GOTO 2010
2190 OPEN A$ FOR OUTPUT AS #1
2200 PRINT #1,M
2210 FOR I=1 TO M
2220 PRINT #1,MM(I)
2230 PRINT #1,N(I)
2240 FOR J=1 TO N(I)
2250 PRINT #1,A(I,J)
2260 NEXT J:NEXT I
2270 CLOSE
2280 GOTO 1970
2290 'O.D. print
2300 LPRINT "O.D. of ";A$:LPRINT :LPRINT
2310 FOR I=1 TO 10
2320 LPRINT "No. ";
2330 LPRINT USING "##";I;:LPRINT "  ";
2340 LPRINT "st.-";:LPRINT USING"##";I;
2350 LPRINT "   C";
2360 LPRINT USING "#.###";W(I);
2370 LPRINT "J ";
2380 FOR J=1 TO N(I)
2390 LPRINT USING "#.###"; A(I,J);:LPRINT " ";
2400 NEXT J
2410 LPRINT
2420 NEXT I
2430 LPRINT
2440 FOR I=11 TO M
2450 LPRINT "No. ";:LPRINT USING "##";I;
2460 LPRINT "  ";L$;"-";
```

Fig. 1. A computer program to calculate ELISA endpoint titer for test sera from their ELISA-ODs obtained at a single dilution.

```
2470 LPRINT USING '###';MM(I);:LPRINT '     ';
2480 LPRINT USING '#.###';A(I,1)
2490 NEXT I
2500 GOTO 1140
2510 'caluculate
2520 LPRINT 'standard curve of [';A$;']'
2530 LPRINT :LPRINT
2540 INPUT'input standard sera 1:c';C
2550 LPRINT'standard sera ---------- 1:';C
2560 INPUT'sample sera 1:d';D
2570 LPRINT'sample sera ---------- 1:';D
2580 INPUT'titer of standard sera ?';E
2590 LPRINT'titer of standard sera--- ';E
2600 INPUT 'cut off st. No.';DD
2610 LPRINT'cut off st.---------- No.';DD
2620 LPRINT :LPRINT :LPRINT
2630 OPEN A$ FOR INPUT AS #1
2640 INPUT #1,M
2650 FOR I=1 TO 10
2660 Q=0
2670 INPUT #1, MM
2680 INPUT #1,N
2690 FOR J=1 TO N
2700 INPUT #1,X(J)
2710 Q=Q+X(J)
2720 NEXT J
2730 M=Q/N
2740 W(I)=M
2750 NEXT I
2760 CLOSE
2770 PRINT CHR$(12)
2780 CLS 2
2790 STD=E*D/C
2800 WINDOW (-.2,-.6)-(LOG(STD)*1.3,1.3)
2810 FOR I=1 TO 9
2820 LINE (LOG(STD*(.5^(I-1))),(1-W(I)))-
     (LOG(STD*(.5^I)),1-W(I+1)),7,,&HF99F
2830 NEXT I
2840 LINE(0,-.5)-(0,1)
2850 LINE(0,1)-(LOG(STD)*1.05,1)
2860 FOR I=1 TO 5
2870 PSET (LOG(10),1-I/200)
2880 PSET (LOG(100),1-I/200)
2890 PSET (LOG(1000),1-I/200)
2900 PSET(LOG(10000),1-I/200)
2910 PSET (I/100,.5)
2920 PSET (I/100, 0)
2930 PSET (I/100,-.5)
2940 NEXT I
2950 LOCATE INT(60*LOG(10)/LOG(STD))+1,22
2960 PRINT '10'
2970 LOCATE INT((60*LOG(100))/LOG(STD)),22
2980 PRINT '100'
2990 LOCATE INT(60*LOG(1000)/LOG(STD)),22
3000 PRINT '1000'
3010 IT=INT(60*LOG(10000)/LOG(STD))
3020 IF IT>76 THEN GOTO 3050
3030 LOCATE INT(60*LOG(10000)/LOG(STD)),22
3040 PRINT '10000'
3050 LOCATE 3,14:PRINT '0.5'
3060 LOCATE 3,8:PRINT '1.0'
3070 LOCATE 3,0:PRINT 'CO.D]'
3080 LOCATE 3,1:PRINT '1.5'
3090 LOCATE 68,19:PRINT '[TITER]'
3100 KEY 1,'':KEY 2,'':KEY 3,''
3101 KEY 4,'':KEY 5,'':
3110 COPY 3
3120 CLS 2
3130 PRINT CHR$(12)
3140 I=0
3150 OPEN A$ FOR INPUT AS #1
3160 INPUT #1,M :U=M
3170 IF EOF(1) THEN CLOSE:GOTO 3300
3180 I=I+1
3190 Q=0
3200 INPUT #1,MM
3210 INPUT #1,N
3220 FOR J=1 TO N
3230 INPUT #1,X(J)
3240 Q=Q+X(J)
3250 NEXT J
3260 M=Q/N
3270 Y(I)=M
3280 M(I)=MM
3290 GOTO 3170
3300 PRINT 'titer of[';A$;']'
3310 LPRINT :LPRINT :LPRINT
3320 LPRINT 'TITERS of [';A$;']'
3330 LPRINT :LPRINT :LPRINT
3340 PRINT '        [code No.]    [O.D.]';
3350 PRINT '      [TITER]'
3360 LPRINT '         [code No.]    [O.D.]';
3370 LPRINT '       [TITER]':LPRINT
3380 FOR I=11 TO U
3390 IF Y(I)<Y(DD) GOTO 3500
3400 IF Y(I)<Y(9) GOTO 3510
3410 IF Y(I)<Y(8) GOTO 3520
3420 IF Y(I)<Y(7)GOTO 3530
3430 IF Y(I)<Y(6) GOTO 3540
3440 IF Y(I)<Y(5) GOTO 3550
3450 IF Y(I)<Y(4) GOTO 3560
3460 IF Y(I)<Y(3) GOTO 3570
3470 IF Y(I)<Y(2)GOTO 3580
3480 IF Y(I)<Y(1) GOTO 3590
3490 VV=1:GOTO 3630
3500 VV=2:GOTO 3630
3510    P=1:GOTO 3600
3520    P=2:GOTO 3600
3530    P=3:GOTO 3600
3540    P=4:GOTO 3600
3550    P=5:GOTO 3600
3560    P=6:GOTO 3600
3570    P=7:GOTO 3600
3580    P=8:GOTO 3600
3590    P=9:GOTO 3600
3600 Y=Y(I)
3610 PP=11-P
3620 X=(LOG((2^P)*2)-LOG(2^P))/(Y(PP-1)-
     Y(PP))*(Y-Y(PP))+LOG(2^P)
3630 PRINT 'NO';I;' ';
3640 PRINT L$;'-';M(I);'    ';
3650 LPRINT 'No.';I;' ';
3660 LPRINT L$;'-';:LPRINT USING '###';M(I);
3670 LPRINT '       ';
3680 IF VV=1 GOTO 3830
3690 IF VV=2 GOTO 3830
3700 Z=EXP(X)
3710 V=Z*D*E/C/1024
3720 VC=INT(V)
3730 PRINT USING '#.###' ; Y(I);
3740 PRINT '     ';
3750 PRINT VC
3760 LPRINT USING '#.###';Y(I);
3770 LPRINT '       ';
3780 LPRINT VC
3790 VV=0
3800 NEXT I
3810 CLOSE
3820 GOTO 1090
3830 PRINT USING '#.###';Y(I);
3840 LPRINT USING '#.###';Y(I);
3850 PRINT '     ';
3860 LPRINT '     ';
3865 A2=E*D/C
3870 IF VV=1 THEN A1$='    >':A1=A2:GOTO 3890
3880 IF VV=2 THEN A1$='    <':A1=A2*(.5^(DD-1))
3890 PRINT A1$;A1
3900 LPRINT A1$;A1
3910 GOTO 3790
```

Fig. 1. (continued).



TITERS of [N-7/15]

	[code No.]	[O.D.]	[TITER]
No. 11	Swine-115	0.291	603
No. 12	Swine-116	0.464	994
No. 13	Swine-117	0.602	1340
No. 14	Swine-118	1.451	> 8000
No. 15	Swine-119	0.845	2231
No. 16	Swine-120	0.849	2249
No. 17	Swine-121	2.000	> 8000
No. 18	Swine-122	0.368	742
No. 19	Swine-123	0.406	840
No. 20	Swine-124	0.550	1198
No. 21	Swine-125	0.194	423
No. 22	Swine-126	1.223	5379
No. 23	Swine-127	1.374	> 8000
No. 24	Swine-128	0.897	2467
No. 25	Swine-129	0.471	1010
No. 26	Swine-130	0.226	500
No. 27	Swine-131	0.040	< 125
No. 28	Swine-132	1.390	> 8000
No. 29	Swine-133	0.550	1198
No. 30	Swine-134	0.225	497

Fig. 2. A standard curve obtained by the computer for serial 2 fold dilutions of a standard positive serum and display of the ELISA titers. The graphical display enables the operator to confirm the validity of the ELISA test.

Table 1. Comparison of the ELISA titer obtained by the computer sistem with that by the graphical method

Specimen Code		ELISA-OD	ELISA titer by	
			computer	graphical
No. 11	KU- 1	0.576	365	360
No. 12	KU- 2	0.605	482	480
No. 13	KU- 3	0.571	351	350
No. 14	KU- 4	0.558	317	320
No. 15	KU- 5	0.557	315	320
No. 16	KU- 6	0.445	159	160
No. 17	KU- 7	0.493	195	200
No. 18	KU- 8	0.564	333	330
No. 19	KU- 9	0.654	813	800
No. 20	KU-10	0.734	>1600	>1600
No. 21	KU-11	0.082	<25	<25
No. 22	KU-12	0.444	159	160
No. 23	KU-17	0.216	51	52
No. 24	KU-14	0.488	191	190
No. 25	KU-15	0.516	229	230
No. 26	KU-16	0.565	335	340
No. 27	KU-17	0.599	452	450
No. 28	KU-18	0.095	<25	<25
No. 29	KU-19	0.683	1215	1200
No. 30	KU-20	0.687	1284	1300

DISCUSSION

Our method of estimating ELISA end point titer from the ELISA-OD at a single dilution is more reproducible and easier to understand compared with the endpoint titration by serial dilution of test sera or showing the titer directly by the ELISA-OD value. Also our method can handle many specimens on a single microplate, although it requires some calculation steps. This step of calculation now became much easier and more rapid by introduction of a computer system. The system of using standard curve was used by Voller *et al.* (1976) to estimate serum factor by the ELISA.

The computer system has another advantage for further statistical analysis of the data for epidemiological studies. For example, the ELISA titer is put into the memory together with other informations, such as age, geographical area, sampling time, and other serological data on the specimen. This procedure will give us correlation coefficients, age distribution, geometrical mean titer and other data very efficiently and rapidly, saving a great amount of time to deal with large numbers of test specimens. We are now planning to connect the computer with ELISA reader so that the OD-value can be directly put into the computer, saving the time of data input.

REFERENCES

1) Ambroise-Thomas, P. & Desgeorges, P. T. (1978a): Diagnostic immuno-enzymologique (ELIS A) des maladies parasitaires par une microméthode modifiée. 1. Modalités techniques. Bull. WHO., 56, 609-613.
2) Ambroise-Thomas, P., Desgeorges, P. T. & Monget, D. (1978b): Diagnostic immuno-enzymologique (ELISA) des maladies parasitaires par une microméthode modifiée. 2. Résultats pour la toxoplasmose, l'amibiase, la trichinose, l'hydatidose et l'aspergillose. Bull. WHO., 56, 797-804.
3) Bundo, K., Matsuo, S. & Igarashi, A. (1981): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. II. Antibody levels in the patient sera. Trop. Med., 23, 135-148.
4) Bundo, K., Morita, K. & Igarashi, A. (1982): enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. III. Assay on antibody titers in swine sera. Trop. Med., 24,87-102.
5) Engvall, E. & Perlman, P. (1971): enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry, 8 , 871-874.
6) Igarashi, A., Bundo, K., Matsuo, S., Makino, Y. & Lin, W-J. (1981): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. I. Basic condition of the assay on human immunoglobulin. Trop. Med., 23, 49-59.
7) Leinikki, P. O. & Passila, S. (1976): Solid phase antibody assay by means of enzyme conjugated to anti-immunoglobulin. J. Clin. Pathol., 29, 1116-1120.
8) Pope, V., Hunter, E. F. & Feeley, J. C. (1982): Evaluation of the microenzyme-linked immunosorbent assay with Treponema pallidum antigen. J. Clin. Microbiol., 15, 630-634.

9) Voller, A., Bidwell, D. E. & Bartlett, A. (1976): Enzyme immunoassays in diagnostic medicine. Theory and practice. Bull. WHO., 53, 55-65.
10) WHO Memorundum (1979): Detection of antigens and IgM antibodies for rapid diagnosis of viral infections. Bull. WHO., 57, 925-930.

日本脳炎ウイルスに対する免疫酵素測定法（ＥＬＩＳＡ）．Ⅳ．被検血清の一点希釈におけるＥＬＩＳＡ吸光度よりＥＬＩＳＡ終末価を算出する為のコンピューターシステムについて

森田公一，分藤桂子，五十嵐　章（長崎大学熱帯医学研究所ウイルス学部門）

従来，半対数グラフ上の検量線から求めていたＥＬＩＳＡ終末価の算出をコンピューターを用いて行なう為のプログラムを作成した．これにより，マイクロ吸光度計より得られる被検血清の一点希釈の吸光度からＥＬＩＳＡ抗体価を求めるまでに要する時間を短縮した．又，この様なデーター処理は疫学統計処理を迅速かつ正確に行なうのに有用である．

熱帯医学　第24巻　第３号　131－137頁，1982年９月