Improvements of a Radioimmunoassay for Measurement of Viral Antibody in Human Sera

WILLIAM R. PATTERSON* AND KENDALL O. SMITH

Department of Microbiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284

Received for publication 14 April 1975

Modifications were made which increased the sensitivity of a solid-phase radioimmunoassay for serum antibody to human herpesviruses. Quantitation of antibody can be accomplished by referring to standard curve titrations.

Radioimmunoassays (RIAs) for quantitation of viral antibody and antigens are being applied in several laboratories (2, 4-6, 8). A system for measuring viral antibodies using beads coated with antigens was described by Smith et al. (10) in 1974. This system has now been improved in sensitivity by two modifications: (i) the reaction times are extended, and (ii) serum to be assayed is diluted in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA) fraction V (Schwarz/Mann, Orangeburg, N.Y.).

Beads (Greiger's of Pasadena, Calif., W773-6) were sensitized with lysates of uninfected and herpes simplex virus type 1 (HSV-1)-infected human fetal lung cells as previously described (10). Each bead was then immersed in 0.5 ml of human serum diluted in twofold increments in PBS with and without 1% BSA (wt/vol). Incubation times of human sera with sensitized beads varied from 30 min to 16 h at room temperature. Serum dilutions were aspirated, and beads were washed 12 times in distilled water by dipping with forceps. Each bead was immersed in 0.5 ml of ^{125}I -labeled guinea pig anti-human globulin and allowed to react for periods from 30 min to 16 h. Fluids were aspirated, beads were washed as before, and radioactivity on the beads was counted in a Beckman Biogamma counter.

A single human serum was titrated several times under different conditions against HSV-1 antigen, and the results are shown in Table 1. Antibody titers are expressed as the reciprocal of the greatest serum dilution which gave counts with virus antigen at least twice those obtained with uninfected cell antigen. An increase in antibody titer was found with increasing time of incubation up to 6 h, at which time both viral antibody and antiglobulin reactions leveled off. For convenience and efficiency, reaction times were fixed at 16 h (overnight) of

exposure to serum and 4 h of exposure to antiglobulin. Addition of 1% BSA to the serum diluent and extending the incubation time boosted antibody titers approximately 32- to 64-fold from values obtained with the 30-min incubation scheme previously described (10). The beneficial effect of BSA was reduction of counts per minute bound to uninfected cell antigen.

Serum antibody titers for HSV-1 obtained by RIA were compared with titers found by microtiter complement fixation (9) and neutralization tests (7). Comparison of titers determined by the three methods showed a good correlation (Table 2). Titers found by RIA averaged about 640 times higher than neutralization titers and 6,000 times higher than complement fixation titers.

Titration of different sera indicated that curves with similar slopes were obtained (Fig. 1) by plotting specific counts per minute (counts per minute obtained with virus antigen-coated beads minus counts per minute obtained with beads coated with uninfected cell antigens) versus reciprocal of serum dilution. This suggested that a standard curve could be constructed, using one serum to which all other sera can be compared. To test this possibility, the curve of serum D (standard curve) was copied onto a piece of tracing paper and superimposed on each point on Fig. 1 obtained in titrations of the other sera. Titers for these other sera (Table 3) were extrapolated from the standard curve (serum D) by reading off the curve the reciprocal of the dilution crossing a line set well above the x-axis, for example, 200 counts/min.

This method compared favorably with the more tedious serial dilution method for B, C, D, and E sera, but not for serum A (a low-titered serum; see Table 4). Serum A had a titer of 100 measured by serial dilution and 1,300 measured by the standard curve method. Higher control counts are frequently observed at low serum

TABLE 1. Effect of length of incubation and 1% BSA on HSV-1 antibody end points obtained by RIA

Serum diluent	Length of incubation	Antibody titer ^a		
		Expt 1	Expt 2	Expt 3
PBS	30 min serum, 30 min antiglobulin	3,200	3,200	6,400
PBS + 1% BSA	30 min serum, 30 min antiglobulin	12,800	ND ^b	ND
PBS	6 h serum, 16 h antiglobulin	25,600	25,600	12,800
PBS + 1% BSA	6 h serum, 16 h antiglobulin	102,400	102,400	ND
PBS	16 h serum, 4 h antiglobulin	6,400	ND	ND
PBS + 1% BSA	16 h serum, 4 h antiglobulin	204,800	128,000	204,800

^a Reciprocal of highest antibody dilution giving >2× counts/min on virus-coated beads compared to counts per minute on control cell beads ($P < 0.001$). Data are presented from several different experiments.

^b ND, Not done.

TABLE 2. Comparison of RIA, complement fixation, and neutralization tests for HSV-1 antibody

Serum	RIA ^a	CF ^b	Neutralization ^c
A	100	<4	<5
B	25,600	8	40
C	51,200	16	80
D	204,800	16	320
E	>204,800	32	320

^a Reciprocal of highest antibody dilution giving >2× counts/min on virus-coated beads compared to counts per minute on control cell beads ($P < 0.001$).

^b Microtiter complement fixation (CF) (9).

^c Eighty percent plaque reduction (7).

TABLE 3. RIA titers obtained with different sera by extrapolating from single points using a superimposed standard curve

Extrapolation from the point at this serum dilution	Antibody titers obtained from standard curve ^a				
	Serum A	Serum B	Serum C	Serum D	Serum E
1:100	1,000	30,000	25,000	150,000	
1:200		40,000	23,000	150,000	
1:400	1,600	36,000	60,000	150,000	
1:800		40,000	75,000	150,000	
1:1,600		40,000	65,000	150,000	
1:3,200		30,000	85,000	150,000	
1:6,400		30,000	58,000	150,000	420,000
1:12,800		25,000	50,000	150,000	330,000
1:25,600		25,000	58,000	150,000	320,000
1:51,200				150,000	300,000
1:102,400				150,000	300,000
1:204,800				150,000	300,000

^a Reciprocal of the highest dilution at which a superimposed standard curve (serum D) dropped below 200 counts/min.

dilutions, making calculation by the 2× control method subject to error on the low side. Therefore, the true antibody titer of a serum would probably be more closely approximated from a standard curve.

The variation within the assay was determined by testing a single serum ten times. A coefficient of variation of 5% was observed in the calculated specific counts (Table 5). The validity of using an end point of 2× virus antigen over control antigen counts was determined by the *t*-variate test (7). For example, 278 counts/min bound to virus antigen at a 1:51,200 dilution was compared to 137 ± 43 counts/min ($\bar{x}$ ± standard deviation) bound to control antigen at 1:6,400 and higher dilutions (Table 6), yielding $t = 8.03$ and $P < 0.001$. When

FIG. 1. HSV-1 serum antibody titrations by RIA. CPM, Counts/min.

TABLE 4. Comparison of RIA titers obtained by standard curve and serial dilution methods

Titer determination method	HSV-1 antibody titer				
	Serum A	Serum B	Serum C	Serum D	Serum E
Standard curve	1,300 $\pm$ 424 ^a	32,888 $\pm$ 6,233	44,166 $\pm$ 18,771	150,000	328,333 $\pm$ 46,654
Serial dilution	100	25,600	51,200	204,800	>204,800

^a Mean $\pm$ standard deviation.

TABLE 5. Variability of RIA

Counts/min bound to control beads ^a	Counts/min bound to HSV-1 beads	Specific counts (virus counts/min minus control counts/min) ^b
654	8,110	7,456
690	8,247	7,556
810	7,983	7,173
679	8,159	7,480
654	7,962	7,318
848	7,932	7,084
860	8,314	7,454
791	9,108	8,317
569	7,752	7,183
858	8,304	7,446

^a A serum was diluted 1:200 and tested ten times.^b Standard deviation, 344; coefficient of variation, 5%; $\bar{x}$, 7,447.

TABLE 6. Titration of a positive serum

Dilution	Counts/min bound to HSV-1	Counts/min bound to control
1:100	11,463	2,379
1:200	10,504	2,137
1:400	6,521	1,424
1:800	5,207	743
1:1,600	3,052	452
1:3,200	1,779	275
1:6,400	958	218
1:12,800	518	140
1:25,600	278	125
1:51,200	206	116
1:102,400	179	131
1:204,800	123	90

determining end points using a standard curve, it is obviously essential to locate one or two points on the more linear portion of that serum titration curve. This can be done for HSV-1 antibody titrations by using dilutions of 1:100, 1:1,000 and 1:10,000 for most human sera.

Sera negative for HSV-1 antibody are rare; two negatives were found in 100 sera tested. An example of a negative serum titration is given in Table 7. Most adult human sera contain antibody levels to HSV-1 that are high and remain constant (3). By this method for RIA,

TABLE 7. Titration of an HSV-1 negative serum

Serum dilution	Control cell counts/min	HSV-1 counts/min
1:10	2,284	2,430
1:50	2,057	1,699
1:100	1,655	1,557
1:300	858	853
1:900	463	359
1:2,700	159	150
1:8,100	163	94
1:24,300	139	86
1:72,900	133	106
1:238,700	161	102
1:716,100	132	104

HSV-1 antibody titers of over 1:100,000 are commonly obtained on sera from humans with chronic herpes simplex lesions. This substantial increase in sensitivity of HSV-1 antibody detection should make it possible to detect extremely low levels of such antibodies, unrecognizable by conventional methods.

This research was aided by Public Health Service grant no. RO1 NS10309 from the National Institute of Neurologic Diseases and Stroke Institute and grant no. In 90E from the American Cancer Society.

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