Application of the Single Radial Diffusion Test for Assay of Antibody to Influenza Type A Viruses

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Single radial diffusion (SRD) tests for antibodies to influenza type A hemagglutinin, neuraminidase, nucleoprotein, and matrix protein antigens were compared with conventional hemagglutination inhibition, neuraminidase inhibition, and complement fixation tests. Sera used in this study were obtained in 1968-1969 from volunteers before and after vaccination and before and after an ensuing epidemic of Hong Kong influenza. The SRD test compared favorably with conventional tests for assessment of vaccine- or infection-induced rises in antibody titers to influenza type A viruses. Little linear relationship was seen between zone areas with SRD and titers with conventional tests, suggesting that the SRD test may detect antibody of different quality or specificity. The SRD seemed equal to the hemagglutination inhibition test for predicting susceptibility to influenza. SRD is a simple test for the recognition of antibody to various antigenic components of the influenza virus and could prove to be a valuable epidemiological tool.

The assay of antibody against influenza viruses is required for serodiagnosis of influenza infections, epidemiological studies of virus spread in the community, and evaluation of vaccine potency and efficacy. Historically, this information has been provided by three basic tests that are generally performed in specialized laboratories: hemagglutination inhibition (HI), neuraminidase inhibition (NI), and complement fixation (CF). Recently a single radial diffusion (SRD) test for the assay of influenza antibodies has been described by Schild et al. (9, 10). This test is based on the formation of a visible reaction between the antigen and its homologous antibody in a supporting agarose gel matrix. It is performed by incorporating the virus antigen into the gel and allowing antisera to diffuse radially from points of application through the fixed antigens. Measurable opalescent zones are produced by the resulting antigen-antibody complexes. The primary advantages of the SRD test are simplicity, economy, and precision in antibody assay. In the present study, the SRD test is compared with HI, NI, and CF tests; the sera used were obtained in 1968-1969 from volunteers before and after influenza vaccination and before and after an ensuing epidemic of Hong Kong influenza.

MATERIALS AND METHODS

Sera. The source of test sera was a vaccine field trial that has been previously described (6, 11). Sera were collected from 217 volunteers before (S1) and 3 weeks after (S2) an injection of either 300 or 3,000 chicken cell agglutinating (CCA) units of monovalent A/Hong Kong/1/68(H3N2), A/Japan/170/62(H2N2), or B/Mass/3/66 vaccines or standard doses of polyvalent vaccine containing A/Japan/170/62(H2N2), A/Taiwan/2/64(H2N2), A/FM/1/47(H1N1), A/PR/8/34(H0N1), and B/Mass/3/66. Three weeks after the vaccination program was completed, a sharp influenza A/Hong Kong (H3N2) epidemic with an attack rate of 40% occurred in this closed population. Three weeks after the epidemic, sera were again collected (S3) and all three sets (S1, S2, and S3) were examined for antibodies by the HI test. NI tests were performed with S1 and S2 serum pairs only. CF tests were performed on the S2 and S3 serum pairs only. These same sera were also examined by the SRD test for the presence of antibodies to the hemagglutinin, neuraminidase, ribonucleoprotein, and matrix (M) protein.

Antigens. The X-31 (5) high-growth-yielding recombinant strain of A/Aichi/2/68(H3N2) and the recombinant A/equine/Prague/1/56(Heq1)-Hong Kong/16/68(N2) were received from E. D. Kilbourne, Mt. Sinai School of Medicine, New York. These antigens are referred to as H3N2 and Heq1N2, respectively. A/chicken/Germany/N/49 (Hav2Neq1) was from the collection of the World Health Organization Collaborating Center for Influenza, London.

Antibody determination: conventional laboratory tests. All sera were stored at -20°C until tested. Standard methods were used to determine HI (3)

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and NI (1) titers. CF tests were done by standard microtiter techniques (7) with nucleoprotein (NP) antigen (8).

SRD tests. Influenza viruses grown in 10-day-old embryonated chicken eggs were concentrated and purified by density gradient centrifugation (13). Virus-agarose suspensions (containing 0.2 mg of purified virus per ml of agarose gel) were prepared and poured onto plastic plates (Hyland, Calif.) as described by Schild et al. (10). By the use of a template, 56 circular wells (2.0 mm in diameter) were cut into the agarose on each plate. Each well held 3 μ l of serum. Test and control sera were added with drawn glass pipettes, and the plates were incubated overnight in a moist chamber.

The presence of antibody was recognized by the appearance of zones of opalescence around the wells. Since accurate measurement of zones directly from plates was difficult, the plates were photographed and prints were made with exact threefold enlargements (10). The diameter of each diffusion area was measured on the photograph, and the actual area was then calculated. The area of the well was subtracted from the final value.

Antibody to the hemagglutinin or neuraminidase, or both, was assayed with intact recombinant H3N2 virus. Antibody to the neuraminidase only was assayed with intact recombinant Heq1N2 virus. Antibody to the Heq1 component of the virus is not found normally in human sera. Antibodies to the internal NP and M protein antigen were assayed with an avian influenza virus A/chicken/Germany/49 (Hav2Neq1) disrupted by sodium Sarkosyl sulfate detergent (10). The surface antigens of this virus are unrelated to those of past or current human influenza viruses. Zones produced by antibody to M protein are more intense than those produced by anti-

body to NP, and this feature was used to distinguish the two antibodies when both were present.

Test criteria. A titer of 10 or greater was considered positive for antibody by the HI test. A titer greater than 100 was considered positive for neuraminidase antibody by the NI test. A titer of 8 or greater was considered positive for antibody by the CF test. A photographed zone of 3 mm² or greater was considered positive for antibody in all the SRD tests.

For the HI and CF tests, a fourfold or greater increase in titer between serum pairs was considered indicative of a significant antibody rise. A threefold or greater increase in titer was considered indicative of a significant antibody rise by the NI test.

An increase of 35% in the area of opalescence in the SRD test was considered to be evidence of a significant antibody rise for all viral antigens used in these tests.

Clinical data. Evaluation methods for influenza-like disease and fever during the epidemic were described in previous publications (11).

Statistical techniques. A binomial test of tabular symmetry (2) was used for each vaccine group to compare the observed number of sera positive by the SRD test with the number positive by the conventional tests. Use of this method is illustrated by analysis of the data in Table 1 for the vaccine category "all groups." The data in this category were used to construct a 2 $\times$ 2 table (Table 2). Whether the number SRD+ (79) is significantly greater than the number HI+ (39) depends upon whether the number SRD+ HI- (61) is significantly greater than the number SRD- HI+ (21). Under the assumption of symmetry, the total number (82) of SRD- HI+ and SRD+ HI- should on the average be evenly distributed between the two groups and

TABLE 1. Results of SRD^a and HI^a tests for antibody to hemagglutinin in single and paired sera

Sera	Vaccine group	No.	SRD+		HI+		Agreement (+ +, --)	
			No.	%	No.	%	No.	%
Detection of anti-body ^b								
S1	All groups	192	79 ^c	41	39 ^c	20	110	57
S2	A (H3N2)	64	59	92	63	98	58	91
S3	A (H2N2)	98	84	86	88	90	80	82
	B	54	42	78	48	89	44	81
Demonstration of antibody rises ^d								
S1-S2	A (H3N2)	60	55	92	53	88	54	90
	B	34	6	18	0	0	28	83
S2-S3	A (H2N2)	96	53 ^c	55	37 ^c	39	72	75
	B	51	31	61	27	53	41	80

^a A/Aichi/2/68 (H3N2).

^b For SRD, test area ≥ 3 mm² is +. For HI test, titer ≥ 10 is +.

^c Number of sera positive by SRD significantly greater than number positive by HI, as shown by a binomial test of tabular symmetry.

^d For SRD, test, ≥ 1.35 -fold area increase is +. For HI test, ≥ 4 -fold titer increase is +.

the probability of observing a split as divergent as 21 and 61 as calculated by the cumulative binomial distribution is:

$$P = \sum_{i=0}^{21} \binom{82}{i} (.5)^i (.5)^{82-i} = 0.002$$

The value of P is less than 0.01; therefore, the observed number SRD+ (79) is considered significantly greater than the number HI- (39). Differences between tests determined to be significant by this technique are noted in Tables 1 through 4.

RESULTS

Detection of antibody to hemagglutinin by SRD and HI. Before vaccination, 41% of sera (S1) from 192 volunteers were found by the SRD test to be positive with A/Hong Kong/68(H3N2) antigen (Table 1). Twenty percent of the sera were positive by the HI test with the same antigen. Agreement between the two tests was poor (57%), since twice as many positives were

TABLE 2. HI and SRD test results: 2 × 2 table

Results	HI test results		Totals
	+	-	
SRD test	+18	61	79
	-21	82	113
Totals	39	143	192

TABLE 3. Results of SRD^a and NI^a tests for neuraminidase antibody in single and paired sera

Sera	Vaccine group	No.	SRD+		NI+		Agreement (++, --)	
			No.	%	No.	%	No.	%
Detection of anti-body ^b								
S1	All groups	182	29	16	37	26	144	79
S2	A (H3N2)	61	41 ^c	67	51 ^c	84	49	80
	A (H2N2)	94	37	39	36	38	55	59
Demonstration of antibody rises ^d								
S1-S2	A (H3N2)	58	38	66	46	79	46	79
	A (H2N2)	89	23	26	25	28	67	75
	B	30	2	7	1	3	29	93

^a A/equine/Prague/1/56(Heq1)-Hong Kong/16/68(N2).

^b For SRD test, area ≥ 3 mm² is +. For NI test, titer in excess of 100 is +.

^c Number of sera positive by SRD is significantly smaller than the number positive by NI, as shown by a binomial test of tabular symmetry.

^d For SRD test, ≥ 1.35 -fold area increase is +. For NI test, ≥ 3 -fold titer increase is +.

TABLE 4. Results of SRD^a and CF^b tests for NP antibody in single and paired sera

Sera	Vaccine group	No.	SRD+		CF+		Agreement (++, --)	
			No.	%	No.	%	No.	%
Detection of anti-body ^c								
S2	All groups	179	142	79	137	77	138	72
S3	All groups	182	166 ^d	91	148 ^d	81	144	79
Demonstration of antibody rises ^e								
S2-S3	A (H2N2)	81	38 ^d	47	20 ^d	25	57	71
	B	44	21	48	15	34	36	82

^a Detergent-disrupted A/chicken/Germany/'N'/49(Hav2Neq1).

^b Purified type A NP.

^c For the SRD test, area ≥ 3 mm² is +. For the CF test, titer ≥ 8 is +.

^d Number of sera positive by SRD is significantly greater than the number positive by CF, as shown by a binomial test of tabular symmetry.

^e For the SRD test, ≥ 1.35 -fold area increase is +. For the CF test, ≥ 4 -fold titer increase is +.

found by the SRD test. By the binomial test of tabular symmetry, the number positive by the SRD test is significantly greater ($P < 0.001$) than the number positive by the HI test.

Three weeks after vaccination, 92% of the sera (S2) from the 64 recipients of the A/Aichi/68(H3N2) monovalent vaccine were positive by the SRD test with H3N2 antigen (Table 1). Ninety-eight percent were positive by the HI test with the same antigen. Agreement between the two tests was high (91%).

The two tests were nearly equal in sensitivity for detecting antibody in the postepidemic (S3) sera from those who did not receive Hong Kong vaccine. The agreements between the SRD and HI test results on the postepidemic sera from the H2N2 and type B vaccine recipients were 82 and 81%, respectively.

Demonstration of rises in antibody to hemagglutinin by SRD and HI. Vaccine-induced antibody rises in paired sera from 60 A/Aichi/68(H3N2) vaccine recipients were demonstrated equally well by the SRD (92%) and the HI (88%) tests with H3N2 antigens (Table 1). Agreement between the two tests was high (90%). No significant rises in HI antibody titers to H3N2 occurred among the type B vaccinees, which demonstrates the specificity of the HI test and the absence of natural influenza infection during the period of vaccination. However, there were six rises in antibody to H3N2 by SRD among the control group members, with the rises equally divided among persons who received low (300 CCA units) and those who received high doses (3,000 CCA units) of type B vaccine.

The ability of the SRD and HI tests to demonstrate antibody rises after natural infection was evaluated with postepidemic paired sera (S2-S3) from recipients of the older type A (H2N2) and type B vaccines (Table 1). Among the H2N2 vaccinees, the SRD test demonstrated a significantly higher number of diagnostic antibody rises (55%) than did the HI test (39%). Among the influenza B vaccinees the number of antibody rises by the SRD test (61%) was slightly higher than that by the HI test (53%), but this difference was not statistically significant.

Detection of antibody to neuraminidase by SRD and NI. The percentage of prevaccination sera with detectable neuraminidase antibody was greater by the NI test (26%) than by the SRD test (16%) when a recombinant antigen Heq1N2 was used (Table 3). With sera of persons vaccinated with A/Aichi(H3N2) vaccine, the proportion positive by the NI test (84%) was significantly ($P < 0.005$) greater than that

positive by the SRD test (67%). With sera of persons vaccinated with A/Japan/62(H2N2) vaccine, the percentage with antibody by the SRD test (39%) was essentially equal to that with antibody by the NI test (38%), but agreement between the two tests was poor (59%).

Demonstration of rises in antibody to neuraminidase by SRD and NI. Vaccine-induced antibody rises in paired sera (S1-S2) from A/Aichi/68(H3N2) vaccine recipients were demonstrated in 38 volunteers (66%) by the SRD test and in 46 volunteers (79%) by the NI test with Heq1N2 antigen (Table 3). This difference was not statistically significant. The frequency of antibody rises among the A/Japan/62(H2N2) vaccinees was lower by both the SRD and NI tests (26 and 28%, respectively). Among the influenza B vaccinees, antibody rises by the SRD test were demonstrated in two serum pairs and by the NI test in one serum pair. None of these serum pairs showed significant antibody rises to H3N2 by the HI test.

Detection of antibody to NP by SRD and CF. Postvaccination (S2) sera from all vaccine recipients were tested for the presence of antibody to the NP antigen by the SRD test with detergent-disrupted A/chicken/Germany/'N'/49-(Hav2Neq1) virus and by the CF test with purified NP antigen (Table 4). The percentage of sera positive by the two tests was similar (79 and 77%, respectively). In postepidemic sera (S3) from all groups, 166 volunteers (91%) had detectable antibody by the SRD test, which was significantly higher ($P < 0.005$) than the 148 (81%) who had detectable antibody by the CF test.

Demonstration of rises in antibody to NP by SRD and CF. In postepidemic sera (S2-S3) the SRD test was significantly ($P < 0.001$) more sensitive than the CF test (47 versus 25%) for demonstrating diagnostic antibody rises among recipients of older A/Japan/62(H2N2) vaccines (Table 4). Similar results were found with postepidemic paired sera from the influenza B vaccinees. The SRD test demonstrated antibody rises in 48% and the CF test in 34% of the serum pairs.

Relationship of SRD and conventional HI, NI, and CF test results. The study data were plotted on scatter diagrams to assess the relationship between SRD zone areas and titers obtained by the HI, NI, and CF tests. These are shown in Fig. 1 through 4. There is little evidence of any linear relationship between SRD and conventional test results. The square of the coefficient of correlation (r^2) for SRD with any test was ≤ 0.33 , where a value of 1 equals perfect linear correlation (14).

FIG. 1. Scatter plot of SRD annulus area versus the associated HI titer; both tests were done with A/Aichi/2/68(H3N2). N, the 64 postvaccination, pre-epidemic sera from vaccine groups H3N2; b, number of sera yielding the same result.

SRD assay of postvaccination and post-epidemic antibody to internal viral proteins. Nineteen to 26% of the postvaccination paired sera from volunteers who received type A vaccines had significant antibody rises by the SRD test when disrupted A/chicken/Germany/'N'/49 virus was used (Table 5). Two of the 44 influenza B vaccinees also showed a significant increase in opalescent zone area. Despite the fact that some of the persons had large doses (3,000 CCA units) of type A vaccines, none of their sera showed patterns typical for M protein antibody. In some postepidemic sera, antibody rises to both internal antigens were observed. Although rises in NP antibody occurred with greater frequency, rises in M protein antibody varied from 2% among the A/Aichi/68

H3N2 vaccinees to as high as 33% among the polyvalent vaccinees. The appearance of M protein antibody was associated with severity of illness as defined by fever, confinement to bed, and mean number of bed days (Table 6). In addition, persons who developed antibody to M protein also developed much higher postinfection CF or HI antibody titers than those who did not. Only one of the severely ill patients who developed M protein antibody had received the A/Aichi vaccine.

Predictive value of pre-epidemic SRD tests for susceptibility to influenza. The SRD test and the HI test with H3N2 antigen were equally useful for assay of probable protective levels of pre-epidemic antibody. Volunteers with no pre-epidemic or postvaccination serum antibodies

FIG. 2. Scatter plot of SRD annulus area versus the associated NI titer; both tests were done with A/equine/Prague/156(Heq1)-Hong Kong/16/68(N2). N, the 40 postvaccination, pre-epidemic sera from vaccine group H3N2; b, number of sera yielding the same results.

by the SRD test had clinical attack rates of 52% for influenza-like disease (Fig. 5) and 46% for influenza-like disease with fever (Fig. 6). Volunteers with postvaccination serum zone ≥ 60 mm² by the SRD test had clinical attack rates of 14% for influenza-like disease (Fig. 5) and 8% for influenza-like disease with fever (Fig. 6). These percentages were basically similar to those observed for HI antibody titers of ≤ 10 and ≥ 320 for the same categories (Fig. 5 and 6).

DISCUSSION

These results document that the SRD test compares favorably with conventional HI, NI, and CF tests for assessment of vaccine- or infection-induced antibodies to influenza. The agreement between test results was generally good. However, the agreement was poor be-

tween SRD and HI test results when the H3N2 antigen was used to detect antibody in pre-vaccination sera and in paired sera from type B vaccinees. The agreement was also poor between the SRD and NI test results when the Heq1N2 antigen was used to detect antibody in sera from H2N2 vaccine recipients.

In the first instance, where the agreement between the SRD and the HI test was 57%, it is unlikely that either test was detecting specific H3 antibodies. The sera were collected before vaccination and before the appearance of the A/Hong Kong (H3) virus. Since the A/Aichi (H3N2) antigen used in the SRD test also contained the N2 component, detection of pre-existing N2 antibody would be expected. The presence of neuraminidase antibody may also explain the occurrence of the small number of HI-positive sera since it is recognized that he-

FIG. 3. Scatter plot of SRD annulus area versus the associated HI titer; both tests were done with A/Aichi/2/68(H3N2). N, the 52 postvaccination, postepidemic sera from vaccine group B; b, number of sera yielding the same results.

FIG. 4. Scatter plot of SRD (with A/Aichi/2/68[H3N2] antigen) annulus area versus the associated CF titer (with purified type A NP). N, the 45 postvaccination, postepidemic sera from vaccine group B; c, number of sera yielding the same results.

magglutination with some virus strains may be inhibited by high concentrations of neuraminidase antibody (12). Some low HI titers may

TABLE 5. Numbers of sera exhibiting significant^a increases in antibody to influenza virus type A NP and M protein as detected by SRD test after vaccination and natural epidemic of A/Hong Kong virus

Vaccine group	Postvaccination (%)		Postepidemic (%)	
	NP	M	NP	M
A (H2N2)	26 (15/57)	0	43 (24/57)	14 (8/57)
A (polyvalent)	21 (5/24)	0	54 (13/24)	33 (8/24)
B	5 (2/44)	0	48 (21/44)	16 (7/44)
A (H3N2)	19 (10/53)	0	24 (13/53)	2 (1/53)

^a Significant increases ($\geq 35\%$) in area of opalescence with detergent-disrupted A/chicken/Germany/'N'/49 (Hav2Neq1).

TABLE 6. Clinical and serological responses of individuals with and without antibody to influenza virus type A M protein after natural infection

M anti-body ^a in serum (S3) (no.)	Clinical influenza (%)	Fever (%)	Confined to bed (%)	Mean no. of bed days per patient	CF		HI	
					Rise (S2→S3) (%)	Mean -fold increase	Rise (S2→S3) (%)	Mean -fold increase
+ (24) ^b	88 (21/24)	83 (20/24)	71 (17/24)	3.0	84 (16/19)	13.0	92 (22/24)	36.0
- (87) ^c	48 (42/87)	43 (37/87)	26 (23/87)	0.77	40 (29/73)	2.5	91 (79/87)	11.6

^a SRD test with detergent-disrupted A/chicken/Germany/'N'/49(Hav2Heq1).

^b Of the 24 subjects, eight received polyvalent vaccine; seven, B vaccine; eight, an H2N2 vaccine; and one, an H3N2 vaccine.

^c All volunteers with serological evidence of infection who failed to develop antibody to matrix protein.

FIG. 5. SRD test (SRDT) and HI titers (A/Hong Kong/68) of postvaccine sera from all vaccines. Relationship of pre-epidemic titers to attack rate based on reported clinical influenza. Numbers in columns are the total vaccinees in each titer category.

also have been caused by incomplete removal of nonspecific inhibitors by pretreatment with receptor-destroying enzyme. However, the rate (41%) of positive prevaccination sera detected by the SRD test with the H3N2 antigen was over twice that (16%) found by the SRD test using the Heq1N2 antigen. The latter antigen was used specifically to detect N2 antibody. The 41% of sera positive by SRD with the H3N2 antigen was also much higher than the 26% found to be positive by the conventional NI test. These results suggest that the SRD test with H3N2 antigen was either more sensitive for neuraminidase antibody than either the SRD or NI test with the Heq1N2 recombinant antigens, or that the SRD detected antibody to some additional antigen. In experimental animals, Schild has observed that antibody resulting from prior experience with other influenza A virus hemagglutinins, namely H2, may pro-

FIG. 6. SRD test (SRDT) and HI titers (A/Hong Kong/68) of postvaccine sera from all vaccinees. Relationship of pre-epidemic columns are the total vaccinees in each titer category.

duce minor cross-reactions with the H3 antigens in this test (G. Schild, unpublished observations); however, a more likely explanation in this study is that antibody resulting from prior immunization with egg-grown vaccines may be reactive with the host antigen incorporated into the virion of the test strain (4).

In further support of the latter explanation is the finding that paired sera from six influenza B vaccinees showed significant increases in zones of opalescence by the SRD test with H3N2 antigen. Since none of the paired sera from influenza B vaccinees had evidence of type A infection by HI test, antibody rises to egg host carbohydrate antigen were strongly considered. By indirect hemagglutination tests with chicken erythrocytes sensitized with concentrates of normal allantoic fluid, 50% of the serum pairs from recipients of the high dose (3,000 CCA units) of B/Mass vaccine showed significant (≥ 4 -fold) antibody rises. Furthermore, 33% of the vaccinees receiving a high dose of B/Mass showed similar diagnostic rises by an indirect hemagglutination test with chicken erythrocytes sensitized with purified egg-grown whole virus recombinant A/equine/Prague/1/56(Heq1)-England/42/72(N2). All increases in agglutinating activity were eliminated by adsorption of these sera with allantoic fluid concentrates before the indirect hemagglutination test was performed, thus providing evidence of antibody to host antigen. Four of the six influenza B/Mass vaccinees with antibody rises by the SRD test also had significant antibody rises by the indirect hemagglutination test with the recombinant virus. Recent experimental studies

with antisera prepared against host antigen suggest that such antisera are capable of producing opalescent zones in the SRD test. However, absorption of these sera with the heterotypic virus before testing in SRD immunoplates containing either influenza A or B virus reveals that antibody to host antigen can be removed. In contrast, antibody to the hemagglutinin cannot be removed by absorption with the heterotypic virus (G. Schild, unpublished observations).

Controls to detect antibody to egg components are clearly needed in the assay of postvaccination sera by the SRD test. Control gels containing allantoic fluids or extracts of chorio-allantoic membranes would have little relevance since the purified virus suspensions used in the SRD test contain a minimum of free host protein. Since purification has no effect on the egg carbohydrate antigen that is incorporated into the virion (4), more appropriate controls might consist of gels containing heterotypic viruses purified in the same manner as the test strain. Even these controls are unlikely to be completely satisfactory because of the variable sensitivity among different virus preparations. For example, the proportion of nonspecific antibody rises detected by SRD tests after vaccination with influenza B was 18% with the A/Aichi antigen, 7% with the recombinant Heq1N2 antigen, and 5% with detergent-disrupted Hav2Neq1 antigen. SRD test results on sera from recently vaccinated populations or populations routinely receiving influenza vaccines should therefore be interpreted with caution.

The explanation for the remaining discrepancy in agreement between the SRD and NI test results with the same recombinant Heq1N2 antigen for detection of antibody in sera from A/Japan/62(H2N2) vaccinees may simply lie in the relative ability of the two tests to detect heterologous antibody of different quality or specificity. This is supported by our finding of the virtual absence of linear correlation between SRD zone size and titers obtained by any of the conventional serologic tests.

In spite of the observed lack of linear correlation, the SRD test with H3N2 antigens would seem to be as accurate as the HI test for predicting susceptibility to influenza. Our results suggest that persons whose sera give SRD zones of ≥ 60 mm² have only one-fifth the risk of infection as those without evidence of antibody. These findings need to be confirmed and extended by testing sera with specific hemagglutinin and specific neuraminidase antigens so that the relationship between SRD test zones and protection against influenza may be more precisely defined.

Another application of the SRD test is for the measurement of M protein antibody. Very little is known of the response of the host to this antigen and the circumstances under which antibody production is initiated. Our results show a clear relationship between rises in M protein antibody and severity of disease. Whether M protein antibody is stimulated as the result of increased virus replication or whether it is associated with a particular pathogenesis of infection remains to be determined.

In summary, the SRD test is a serological technique that can be used for the detection of antibody to various components of the influenza virus. A disadvantage of the test is that highly concentrated stocks of virus are required, and thus the preparation of plates may not be within the capability of many virus laboratories. On the other hand, if the SRD plates can be provided by a central laboratory, the advantages of the test are numerous. It can be performed by persons with minimal training. It is simple and rapid. There are no problems with nonspecific inhibitors, and, therefore, no pretreatment of sera is required. A large number of sera can be tested on a single plate, and a reliable, permanent, photographic record can be made. Although the standard serological tests may remain the choice for specialized laboratories capable of performing and interpreting them, the SRD test offers smaller hospital and epidemiological laboratories the oppor-

tunity and ability to perform diagnostic tests for influenza.

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