Single-radial-haemolysis: a new method for the assay of antibody to influenza haemagglutinin

Applications for diagnosis and seroepidemiologic surveillance of influenza

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A simple single-radial-haemolysis technique is described that permits the detection and assay of antibody to influenza virus haemagglutinin. The method depends on the passive haemolysis of virus-treated erythrocytes by antihaemagglutinin antibody and complement. Under the "standard" test conditions described antibody to the other surface antigen of the influenza virus (neuraminidase) or to the internal antigens of the virus (nucleoprotein and matrix protein) do not produce haemolysis. Because it requires only small amounts of crude virus antigen and is rapid and simple, the method appears to be of considerable value for large-scale seroepidemiologic studies of new influenza virus variants. Antihaemagglutinin antibody detected by single-radial-haemolysis appears to be relatively strain-specific; the technique may therefore be useful in the antigenic characterization of virus isolates.

Influenza epidemics occur almost annually and antigenic variants of influenza virus appear frequently. It is important that information on the immune status of the population to such strains be kept constantly under review in order to provide a basis for recommendations on vaccination. There is therefore a considerable need for simple and rapid laboratory tests for antibodies to influenza.

In the past the haemagglutination-inhibition (HI) test has been widely used, but this technique has certain drawbacks which arise from the presence of nonspecific inhibitors in serum and from variations in the characteristics of the virus and erythrocytes used. An alternative test, which is simple to perform and avoids the disadvantages of the HI test, is the single-radial-diffusion test (1). This method was satisfactory for seroepidemiologic surveys of influenza (2), but it requires relatively large amounts of purified or semipurified influenza virus as antigen. Whereas this is not a problem for established variants which may be obtained as high yielding recombinants (3), it may cause difficulties in the case of new variants for which serologic information is urgently required.

In this paper we describe a new method based on the haemolysis of erythrocytes, which are sensitized by "coating" with influenza virus particles, by specific antihaemagglutinin antibody and complement. For convenience the method was adapted as a modified single-radial-diffusion test, i.e., single-radial-haemolysis (SRH). SRH requires only small amounts of influenza virus and can therefore be performed with infected allantoic fluids from recently isolated influenza viruses without the need to adapt the virus to rapid growth. This feature has made the test particularly useful for the rapid screening of antibody against newly detected influenza variants. In the present study this technique was compared with haemagglutination-inhibition for a survey of antibody to the haemagglutinins of 4 influenza A variants (A/Port Chalmers/1/73 (H3N2), A/Hannover/61/73, A/Puerto Rico/1/74, and A/England/635/74) that have recently appeared in various countries.

MATERIALS AND METHODS

Virus strains

These were obtained from the collection of the WHO World Influenza Centre or from the Virus Reference Laboratory. The strains A/Port Chalmers/1/73 (H3N2), A/Hannover/61/73 (H3N2),

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A/Puerto Rico/1/74 (H3N2), and A/England/635/74 (H3N2) were recent isolates with a low number of serial passages (3–5) in the egg allantoic cavity. The origins of these viruses are described elsewhere (4). Recombinant viruses between A/Hong Kong/1/68 (H3N2) and A/equine/Prague/56 (Heq1 Neq1), i.e., H3 Neq1 and Heq1 N2, were supplied by Dr E. D. Kilbourne, Mt Sinai Hospital Medical School, New York, USA.

Antisera

Postinfection ferret serum was obtained 12–14 days after intranasal infection of the animals with egg-grown influenza virus. Antiviral serum was prepared in guinea-pigs or rabbits injected intramuscularly with 300 μg of purified influenza virus and was collected after 4–6 weeks. Antiserum to specific antigenic components was prepared in rabbits or guinea-pigs hyperimmunized with purified haemagglutinin or neuraminidase preparations as described previously (1, 5, 6, 7). Antisera to influenza A ribonucleoprotein and matrix protein antigens were prepared as described by Schild and Pereira (8) and Schild (9).

Human serum

Serum was collected from " normal " adults aged 17–45 years in London during November, 1974. Paired serum samples were obtained from adults with naturally acquired influenza A and B infections in early 1974. Dr J. W. Smith, Public Health Laboratory, Colindale, supplied serum from vaccinees who had received A/Port Chalmers/1/73 inactivated vaccine.

Haemagglutination-inhibition (HI) tests

These tests were performed as described by Pereira et al. (10).

Preparation of SRH immunoplates

Ten per cent (v/v) suspensions of freshly washed chicken erythrocytes were made up in phosphate buffered saline (PBS) at pH 7.0–7.2 (11). Influenza virus was added to the erythrocytes in the form of purified virus or crude allantoic fluid at a " standard " concentration of 500 haemagglutinating units (HAU) per ml of 10% erythrocyte suspension. The suspensions were held at 4°C for 10 min in order to allow the virus to adsorb to the erythrocytes; unadsorbed virus was then removed by two cycles of sedimentation by low speed centrifugation and resuspension in fresh PBS. When crude allantoic fluids were used control erythrocytes were treated with an equivalent volume of uninfected allantoic fluid. Immunoplates were prepared by incorporating 0.3 ml of virus-treated erythrocytes together with 0.1 ml of complement (undiluted guinea-pig serum) in 2.6-ml volumes of melted agarose held at 45°C in a water bath. The final concentration of complement in the gel was equivalent to 2.5 minimum haemolytic doses in a standard assay system with sheep erythrocytes (12). The agarose used was A-37 Indubiose (l'Industrie biologique française, Gennevilliers, France) made up to contain 1.5% agarose in PBS at a final pH of 7.0–7.1; 0.1% sodium azide was added as a preservative. The agarose was melted at 100°C and cooled to 42°C before use. The 3.0-ml volumes of erythrocyte suspension in agarose were shaken vigorously to distribute the erythrocytes evenly and were then poured into empty plastic immunoplates (microscope-slide-sized chambers from Hyland Laboratories, Costa Mesa, CA, USA). Wells 2 mm in diameter were cut in the gel after the plates had been left for 30 min to allow the agarose to set. Prepared immunoplates may be stored at 4°C for up to 48 h before use.

SRH test procedure

The serum was maintained at 60°C for 20 min before it was added to the SRH immunoplates. Five-microlitre volumes of undiluted or 1 : 10 serum were added to the wells in the immunoplate and the plates were then transferred to a humid environment and incubated at 37°C for 16 h before they were read. The diameters of the zones of complete or partial haemolysis that developed around the wells were measured with a micrometer eyepiece scale calibrated in 0.1-ml divisions (Matchless Machines, Horsham, England). Each serum sample was introduced into two wells and the mean zone diameter of the responses was calculated; however, there were only minor variations between replicate tests.

Analysis (Schild, unpublished data) of the reproducibility of the test indicated that differences in zone diameter as low as 10% were statistically valid and permitted differences of antibody potency as small as 50% between serum samples to be detected with significance. This is a somewhat lower degree of reproducibility than that afforded by single-radial-diffusion tests for influenza (5, 6), but it allows smaller differences in antibody level to be detected than is possible by conventional HI tests. For HI tests only changes of 400% or greater are regarded as being significant.

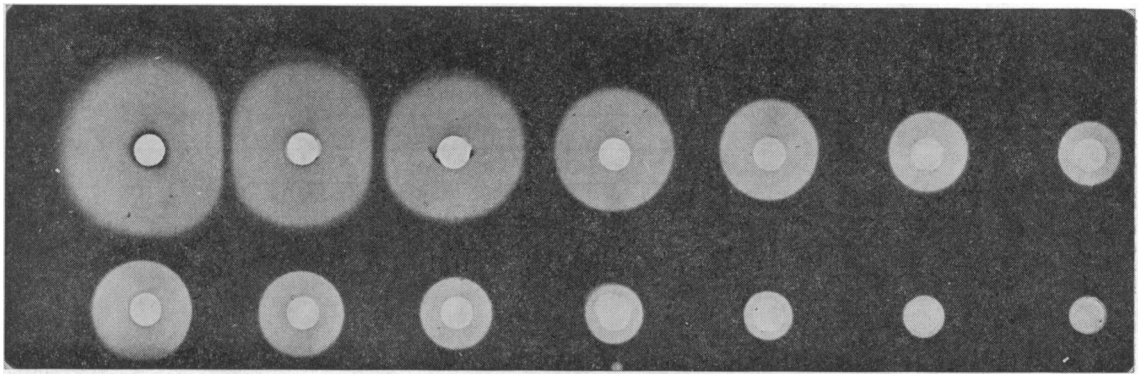

Fig. 1. Single-radial-haemolysis reactions of human serum in agarose gel immunoplates containing guinea-pig complement (final concentration 1 : 30) and chicken erythrocytes (1%) treated with A/Port Chalmers/1/73 virus. The clear areas represent zones of lysed erythrocytes produced by antibody to haemagglutinin. The wells in the top row contain serial, two-fold dilutions (1 : 1 to 1 : 64) of a potent human serum having an HI titre of 1 : 2 560 with A/PC/1/73 virus. The bottom row contains similar dilutions of a serum with an HI titre of 1 : 256.

As a control for the presence of nonspecific haemolytic activity each serum sample was tested on immunoplates containing complement and unsensitized erythrocytes or erythrocytes treated with normal allantoic fluids. Such nonspecific haemolysis may be due to the presence of noninactivated bacterial haemolysins or to the presence of antibodies reacting with chicken erythrocytes (e.g., Forssman antigens). In the latter case nonspecific activity is eliminated by absorption of the serum with chick erythrocytes before testing. In the present studies fewer than 1% of the human serum samples gave nonspecific reactions.

In some studies the specificity of the SRH reaction for specific antiviral antibody was established by the absorption of test serum with preparations of purified, intact influenza virus as described previously (5, 6). The specificity of the SRH reactions was demonstrated by the complete removal of SRH activity by absorption with virus homologous to that used in the SRH immunoplate.

RESULTS

Dose response and specificity of SRH reactions

Human convalescent serum from individuals with recent influenza A infections was found to give a potent SRH reaction. Fig. 1 illustrates the reactions of a series of dilutions (from 1 : 1 to 1 : 64) of two convalescent serum samples from individuals with laboratory confirmed influenza A/Port Chalmers/1/73 (H3N2) infections in SRH tests with chicken erythrocytes treated with the homologous virus. The HI titres of these samples were 1 : 2 560 (row A) and 1 : 256 (row B). The size of the zones of clear haemolysis surrounding wells containing antiserum decreased regularly with increasing serum dilution. A plot of $\log_{10}$ zone diameter against $\log_2$ serum dilution for samples of different antibody potencies was linear and gave parallel plots for samples of different potencies (Fig. 2). These samples

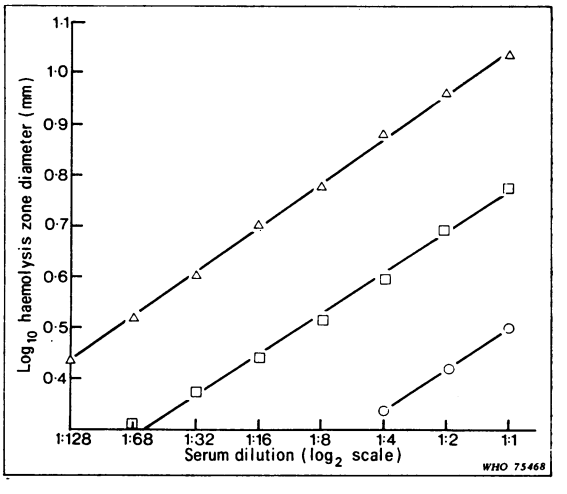

Fig. 2. Dose response curves of serial dilutions of 3 samples of convalescent human serum of different HI antibody potencies. Triangles represent a sample with an HI titre of 1 : 2 560, squares a sample with a titre of 1 : 256, and circles a sample with a titre of 1 : 20. A plot of $\log_{10}$ zone diameter against serum dilution ($\log_2$ scale) gave parallel straight line relationships for each of the samples.

Table 1. Specificity of the single-radial-haemolysis test. Lack of cross-reactivity between different influenza A virus subtypes

Virus strain	Diameter of zone of clear haemolysis (mm) antiserum prepared against:							
	A/PR8/34		A/FM1/47		A/Sing/57	A/HK/68		B/Lee/40
	F[a]	GP[b]	F	GP	F	F	GP	F
A/PR8/34 (H0N1)	7.3	8.5	—	—	—	—	—	—
A/FM1/47 (H1N1)	—	—	6.9	9.0	—	—	—	—
A/Singapore/1/57 (H2N2)	—	—	—	—	6.5	—	—	—
A/Hong Kong/1/68 (H3N2)	—	—	—	—	—	8.2	9.3	—
B/Lee/40	—	—	—	—	—	—	—	7.1

[a] F = results obtained with postinfection ferret serum.
[b] GP = results obtained with immune guinea-pig serum.

failed to produce SRH reactions in immunoplates containing appropriately " sensitized " erythrocytes but no complement, or in plates containing complement plus erythrocytes treated with normal, uninfected allantoic fluids. Although 500 HAU of virus per ml of 10% erythrocyte suspension was used routinely, positive reactions were detected over a wide range of virus concentrations, 50–10 000 HAU. However, with a low virus concentration, 50–100 HAU, the zones were of partial haemolysis in which some of the erythrocytes remained unlysed.

The serum of guinea-pigs or rabbits immunized with preparations of highly purified, intact influenza virus gave a potent SRH reaction with similar dose response characteristics to those shown by human serum. In order to establish the specificity of the antibody responsible for this reaction, antiserum from guinea-pigs and rabbits immunized with purified antigenic components of influenza virus was tested. Antiserum to purified haemagglutinin isolated from A/Port Chalmers/1/73 or A/Hong Kong/1/68 gave potent SRH reactions in immunoplates containing the homologous virus strain used at a " standard " concentration of 500 HAU per ml of 10% erythrocytes. In such immunoplates no haemolysis was detected with potent antiserum prepared in rabbits or guinea-pigs against purified neuraminidase antigen (N2) of A/Hong Kong/1/68 (neuraminidase-inhibition titre 1 : 3 000) or with potent antiserum prepared against the two major internal antigens of the virus, i.e., the nucleoprotein and the matrix protein.

Further evidence that with a standard virus concentration the SRH reaction is dependent on antibody to the haemagglutinin antigen was obtained by the use of recombinant influenza viruses. Two " reciprocal " recombinant influenza virus strains derived from A/Hong Kong/1/68 (H3N2) and A/equine/Prague/56 (Heq1 Neq1) as parents were used; these were of antigenic composition H3 Neq1 and Heq1 N2. Guinea-pig antiserum to purified A/Hong Kong/1/68 (H3N2) reacted with equal potency to erythrocytes sensitized with A/Hong Kong/1/68 and to the N3 Neq1 recombinant, but failed to react to the Heq1 N2 recombinant. In contrast, antiserum to the equine virus Heq1 Neq1 reacted to the Heq1 N2 recombinant but not to H3 Neq1. However, when high concentrations of virus (3 000 HAU per ml of 10% erythrocytes) were used guinea-pig and rabbit antineuraminidase serum produced zones of partial haemolysis which were not present at lower virus concentrations. Such zones were extremely diffuse and difficult to discern but were nevertheless reproducible with antineuraminidase serum. Antibodies to the internal components of the virus did not give SRH reactions even when such high concentrations of virus were used.

In order to determine the specificity of the SRH reaction for influenza viruses containing haemagglutinins of distinct subtypes, immunoplates containing standard concentrations (500 HAU) of influenza A virus subtypes H0, H1, H2, and H3 and of B/Lee/40 virus were prepared, and the SRH reactions of postinfection ferret serum or immune guinea-pig serum were tested. The results are shown in Table 1. For both ferret and guinea-pig serum the

Table 2. Strain specificity of single-radial-haemolysis reactions

Virus strain	Zone diameter (mm) antiserum prepared against: A/HK/68 F[a]	A/HK/68 GP[b]	A/Eng/72 F	A/PC/73 F	A/PC/73 GP	A/Hann/73 F	A/PR/74 F	A/Eng/74 F
A/Hong Kong/1/68	8.2c[c]	9.3c	5.5c	—	—	—	—	<3.0
A/England/42/72	5.5p[d]	5.7p	8.4c	4.3c	6.3c	3.3p	3.6p	3.4p
A/Port Chalmers/1/73	—	3.5p	6.5c	8.6c	10.2c	6.0c	5.3p	6.3c
A/Hannover/61/73	—	—	3.6p	5.3p	6.5c	7.2c	5.3p	7.0c
A/Puerto Rico/1/74	—	—	3.5p	4.8p	4.9p	3.8p	8.9c	3.7p
A/England/635/74	—	—	—	3.5p	3.2p	5.2p	4.8p	8.9c

[a] F = postinfection ferret serum.
[b] GP = immune guinea-pig serum.
[c] Figures followed by the letter c represent diameters of zones of clear haemolysis.
[d] Figures followed by the better p represent diameters of zones of partial haemolysis.

haemolysis reactions were specific for each haemagglutinin subtype and no cross-reactions were detected. Table 2 shows the cross-reactions detected in SRH tests of a number of viruses of the H3N2 subtype, including A/Hong Kong/1/68 and strains isolated at various times up to 1974. These tests provided clear evidence of antigenic differences between the test strains; the zone areas of the heterologous reactions were considerably smaller than those of the homologous reaction. Whereas homologous reactions produced zones of clear haemolysis, heterologous reactions often resulted in zones of partial haemolysis. Cross-reactions between strains were not always detected despite the fact that the test isolates were all of the H3N2 subtype; for example, antiserum to A/Hong Kong/1/68 failed to react with strains isolated in 1973 or 1974. In general, the SRH test showed a higher degree of strain specificity than has been reported for HI tests with the same strain (4).

Performance of SRH for serologic surveillance and serodiagnosis

In order to assess the value of SRH tests for seroepidemiologic surveys, 258 serum samples from normal adults were assayed for antibody by both HI and SRH tests. The variant prevalent at the time, A/Port Chalmers/1/73, and 3 more recent strains isolated in different areas of the world, A/Hannover/61/73, A/Puerto Rico/1/74, and A/England/635/74, were used. The results obtained with a representative number of samples are shown in Fig. 3 by a plot of HI titre against the SRH zone diameter produced by undiluted serum. In general, a close correlation between the antibody potencies measured in both test systems was observed; serum samples with high HI titres (1 : 2 560–1 : 5 120) gave zone

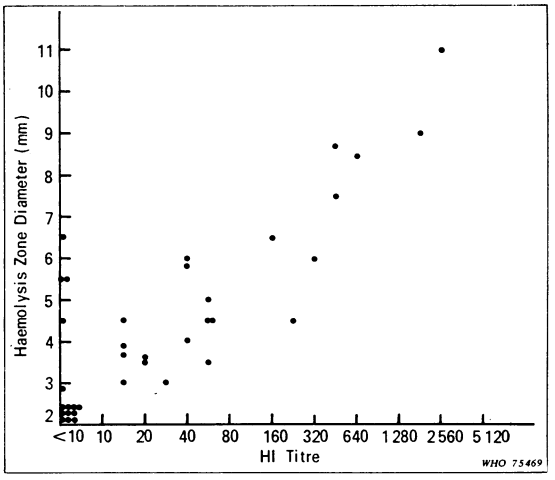

Fig. 3. Correlation between HI titre and SRH zone diameter for A/Port Chalmers/1/73 (H3N2) virus in 37 human serum samples. The samples were collected from adults aged 17–45 years in London in November, 1974.

diameters of 9–11 mm whereas those with HI titres of 1 : 160–1 : 320 gave diameters of 4.5–6.5 mm. Of 15 samples shown in Fig. 3 that were negative in HI tests (titre <1 : 10) 10 were also negative by SRH; the remaining 5 samples gave positive SRH reactions. The detection of responses by SRH in the absence of a detectable HI reaction is of doubtful significance, but the SRH reactions were found to be virus-specific and no reactions were detected on control immunoplates without viral antigen.

A summary of the proportion of samples that gave positive SRH reactions (those that gave a zone diameter of ⩾2.5 mm when tested undiluted were regarded as positive) and HI titres of ⩾1:20 against the various test viruses is shown in Fig. 4. It can be seen that a very similar distribution of antibody was detected by the two tests. Antibody to A/Port Chalmers/1/73 was detected with both tests in approximately 30% of the samples, whereas with A/Hannover/61/73 and A/Puerto Rico/1/74 a somewhat higher proportion of the samples were positive. With the strain A/England/635/74, which in tests with ferret antiserum appeared to have undergone a greater degree of antigenic " drift " away from A/Port Chalmers/1/73 than had the other two variants (Table 1) (4), antibody was detected in a lower proportion of the samples (25% by HI and 18% by SRH).

Preliminary studies were made to evaluate the diagnostic value of the SRH test and its ability to detect influenza vaccine-induced antibody (Table 3). Paired serum samples from 11 individuals with clinical A/Port Chalmers/1/73 infections that had been confirmed by virus isolation were assayed by both HI and SRH. All individuals showed 4-fold or greater rises in HI titre and significant increases in SRH zone diameters against the homologous test virus. No increases in antibody were detected with B/Hong Kong/8/73 virus in SRH tests. Paired samples from 3 persons with influenza B infections showed increases in SRH antibody to B/Hong Kong/8/73 but no response to A/Port Chalmers/1/73. Antibody responses to inactivated A/Port Chalmers/1/73 vaccine were readily detected by SRH tests with the homologous virus and paralleled the increases in antibody detected by HI tests. No increase in SRH activity was detected with influenza B test virus.

Convalescent serum obtained from individuals either following A/Port Chalmers/1/73 infection or following vaccination with inactivated Port Chalmers/73 vaccine (Table 3) were adsorbed by the addition of concentrates of A/Port Chalmers/73 as

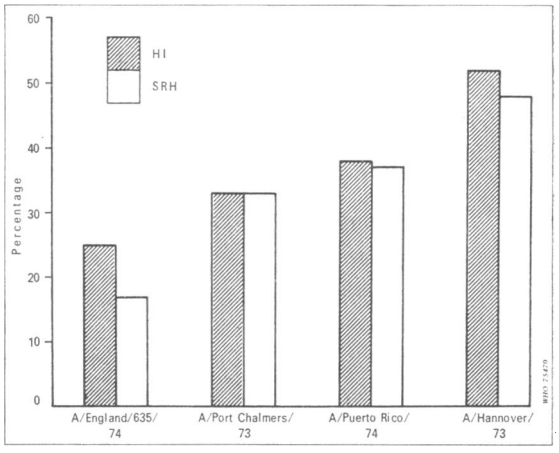

Fig. 4. Comparisons of the frequency of detection of antibody by HI tests (HI titre 1:20) and SRH (zone diameter ⩾ 2.5 mm) to 4 different influenza A (H3N2) variants in 221 human serum samples. The source of human serum was the same as that for Fig. 3.

described by Schild et al. (5, 6). Adsorbed serum failed to give SRH reactions in immunoplates containing erythrocytes treated with A/Port Chalmers/73 virus, thus indicating the virus-specific nature of the SRH reaction.

DISCUSSION

Although passive agglutination by antibodies of erythrocytes with microbial antigens attached to their surfaces has been used frequently in studies of antibodies to microbial antigens (reviewed by Herbert, 13), passive haemolysis techniques have not been widely exploited. The present paper describes the passive antibody- and complement-mediated lysis of erythrocytes sensitized with influenza virus and the application of this phenomenon to the assay of specific antibody to influenza haemagglutinin.

Although the parameters of the test have not yet been fully investigated, it is clear from the data presented that the SRH method is of considerable potential value for the serologic diagnosis and surveillance of influenza. Of special merit are the simplicity and convenience of a test that may be performed with undiluted and untreated serum, its accuracy and reproducibility, and its freedom from the effects of nonspecific inhibitors of haemagglutination that are troublesome in conventional HI tests. Moreover, SRH tests employing the " standard " concentration of virus described in this paper

Table 3. Comparisons of antibody responses by SRH and HI tests in paired sera from individuals with naturally occurring influenza A and influenza B infections and in recipients of A/Port Chalmers/1/73 vaccine

Paired sera from:	Test antigen					
	A/Port Chalmers/1/73				B/Hong Kong/8/73	
	HI		SRH			
	a[a]	c[a]	a	c	a	c
A/Port Chalmers/73 infections	60[b]	1920	5.2[c]	8.9	5.4[c]	5.5
	15	480	3.0	7.5	0	0
	<10	160	0	5.5	3.5	3.7
	60	5120	5.3	8.7	0	0
	15	960	0	8.0	0	0
	<10	240	0	6.5	0	0
	15	960	5.9	8.5	4.3	4.3
	<10	120	0	6.8	5.4	5.5
	<10	960	0	7.0	0	0
	<10	60	0	5.5	0	0
	<10	480	0	7.4	0	0
B/Hong Kong/73 infections	<10	<10	0	0	0	4.8
	<10	<10	0	0	0	7.5
	60	80	4.5	4.5	0	6.3
A/Port Chalmers/73 vaccinees[d]	20	480	3.5	6.7	0	0
	<10	960	0	8.3	3.0	3.0
	80	1920	5.5	9.5	0	0
	<10	480	0	5.8	0	0
	<10	5120	0	11.3	4.3	4.5

[a] a and c represent acute and convalescent serum samples, respectively, taken at the onset of infection and 2-3 weeks postinfection (for vaccinees prevaccination and 6 weeks postvaccination).

[b] Reciprocal HI titres.

[c] SRH zone diameter with undiluted serum samples.

[d] Vaccinees received a monovalent, inactivated vaccine containing 400 IU of A/Port Chalmers/1/73 virus.

(500 HAU per ml of 10% erythrocytes) are specific for antibody to haemagglutinin and it is this antibody that plays a major role as a serologic index of immunity and past exposure to influenza infection.

It is not clear why antibodies to the other surface antigen of the influenza virus, the neuraminidase, did not give SRH responses under "standard" test conditions; they give very feeble haemolysis with higher virus concentrations, and further investigations on the mechanism of the test are required. Concentrated virus preparations are not required for SRH tests and a single allantoic passage of virus in a small number of eggs provides sufficient antigen for several immunoplates, whereas the single-radial-diffusion test described previously (2) requires relatively purified and concentrated virus. The SRH test is relatively strain-specific and offers an additional method for the antigenic characterization of influenza variants.

Antibody responses to natural infection and vaccination are readily detected by SRH tests. Preliminary investigations on the nature of the antibody

that is detected by SRH tests indicated that in serum samples collected 3–6 weeks after infection or immunization it is essentially IgG antibodies that are detected by SRH. Haemolytic activity may be completely eliminated by treating test serum with antiglobulin serum to IgG but not with antiserum to IgA or IgM (Schild, unpublished data). In addition, antibodies detected by SRH after natural influenza infection have been found to persist with only a small reduction in titre for up to 12 months.

For large scale serologic surveys SRH offers the advantages of rapidity and simplicity in use. In the survey here described it is of interest that following the influenza outbreaks of 1974–75 only 30% of individuals tested possessed antibody to the then prevalent variant A/Port Chalmers/1/73 and an even smaller proportion had antibody to A/England/635/74, indicating that the population was largely susceptible to these viruses during the influenza season 1974–75.

SRH methods are potentially applicable to serologic studies on a wide range of viruses of medical interest, particularly those that have a natural affinity for the erythrocyte surface. Preliminary studies with rubella virus (Schild and Bradstreet, unpublished data) have shown that the SRH method is effective for antibody assays and for the serologic diagnosis of infection with paired serum samples. In addition, it is likely that agents that do not naturally attach to erythrocytes may nevertheless be coupled to the cell surface by chemical means (13), thus allowing SRH tests to be performed.

RÉSUMÉ

L'HÉMOLYSE RADIALE SIMPLE, NOUVELLE MÉTHODE DE TITRAGE DE L'ANTICORPS ANTI-HÉMAGGLUTINE DU VIRUS GRIPPAL. APPLICATIONS AU DIAGNOSTIC ET À LA SURVEILLANCE SÉRO-ÉPIDÉMIOLOGIQUE DE LA GRIPPE

Le présent article décrit une technique facile d'hémolyse radiale simple permettant de détecter et de titrer l'anticorps anti-hémagglutinine du virus grippal. Cette méthode est basée sur l'hémolyse passive, par l'anticorps anti-hémagglutinine et le complément, d'érythrocytes traités par le virus. Dans les conditions d'épreuve « standard » décrites, les anticorps contre l'autre antigène de surface du virus grippal (neuraminidase) ou contre les antigènes internes de ce virus (nucléoprotéine et protéine interne) ne provoquent pas d'hémolyse. Cette méthode, qui n'exige que de petites quantités d'antigène viral brut et qui est rapide et simple, paraît d'un intérêt considérable pour les études séro-épidémiologiques à grande échelle des nouveaux variants du virus grippal. L'anticorps anti-hémagglutinine détecté par l'hémolyse radiale simple semble relativement spécifique de souche, et cette technique peut donc être précieuse pour la caractérisation antigénique des virus isolés.

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