Radioimmunoprecipitation Assay for Quantitation of Serum Antibody to the Hemagglutinin of Type A Influenza Virus

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A double-antibody radioimmunoprecipitation (RIP) assay has been developed to provide a sensitive and specific measure of antibody to hemagglutinins of H3N2 influenza viruses. Chloramine T was used to radiolabel purified hemagglutinins to high specific activity without loss of antigenicity. The purity of the labeled hemagglutinin was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, which also established that both the HA₁ and HA₂ polypeptides were iodinated. Radiolabeled hemagglutinins with a specific activity that did not exceed 12 $\mu\text{Ci}/\mu\text{g}$ of protein could be maintained for up to 30 days at -70°C in the presence of supplemental protein. The RIP assay was compared with conventional methods, hemagglutination inhibition and viral neutralization tests, using H3N equine 1 hybrid viruses for determining serum antihemagglutinin antibody titers. The geometric mean titers for human convalescent sera after infection with A/England/72 virus were 118, 161, and 18,822 for hemagglutination inhibition, viral neutralization, and RIP tests, respectively, and the three tests demonstrated significant rises in antihemagglutinin antibody titers with equal efficiency. In general, a positive correlation existed between antihemagglutinin antibody titers determined by these three procedures; however, the antibody level determined by RIP assay for each individual could not be related to hemagglutination inhibition or viral neutralization titers by a constant factor. A similar lack of a constant relationship was found by using hyperimmune guinea pig antisera, which suggests that the RIP assay can detect antibody populations that exhibit differing efficiencies for inhibition of viral hemagglutination and replication.

Radiolabeling techniques are powerful immunological tools for quantitative determinations of viral antigens and antibodies (1). Whole viruses, antigenic hybrid viruses, and soluble glycoprotein products obtained by bromelain cleavage of intact viruses have been used as test antigens for the assay of antihemagglutinin antibody (AHAb) to influenza viruses (3, 10, 14). In addition, solid-phase radioimmunoassays (RIA) have been employed for the same purpose (4, 5, 15). This report describes a double antibody radioimmunoprecipitation (RIP) assay which utilizes iodinated preparations of purified hemagglutinin (HA) subunits of H3N2 viruses. In addition, data are presented on the sensitivity of the assay for detection of viral antigen and antiviral antibody.

MATERIALS AND METHODS

Source of HA subunits. H3N2 HA subunits derived from strains representative of A/Hong Kong/68 (A/HK/68), A/England/72 (A/Eng/72), A/Port Chalmers/73 (A/PC/73), and A/Scotland/74 viruses were purified as previously described (8). These materials

were provided by the Development and Applications Branch of the National Institute of Allergy and Infectious Diseases.

Animal antisera. Antisera to the purified HA and to purified whole viruses were raised in young adult female guinea pigs. HA (50 μg) or whole virus (200 μg) in saline was emulsified with an equal volume of Freund complete adjuvant. Guinea pigs were inoculated both in the footpads, at 0.1 ml per footpad, and intramuscularly, 0.1 ml in each hip. Approximately 1 month later, the guinea pigs were reinoculated intramuscularly with the same dose in Freund incomplete adjuvant. The animals were bled by cardiac puncture 14 to 21 days after the booster inoculation.

Iodination of the HA. Purified HA subunits were iodinated by a modification of the Greenwood et al. method (7). For labeling, the reaction mixture consisted of the following reagents: 30 μl of 0.5 M sodium phosphate buffer (pH 7.2), 20 μl of HA (20 to 40 μg of protein) in phosphate-buffered saline, 10 μl of Na¹²⁵I (Amersham/Searle Corp., Arlington Heights, Ill.) containing 1 mCi of radioactivity, and 10 μl of chloramine T (5 mg/ml; Matheson, Coleman, and Bell, Norwood, Ohio). After incubation at room temperature for 30 s, the reaction was stopped by addition of 10 μl of sodium metabisulfite (10 mg/ml) followed by addition of 100

μ l of KI (1 mg/ml). Unbound radioactivity was removed by chromatography on a Sephadex G-100 column. The radiolabeled HA eluted in the void volume; these fractions were pooled, fetal calf serum was added to a final concentration of 25%, and appropriate volumes were stored at -70°C . The specific activity of antigens iodinated by this procedure was 7.3 to 10.9 $\mu\text{Ci}/\mu\text{g}$, and greater than 95% of the radioactivity was precipitable by 10% trichloroacetic acid.

Polyacrylamide disc gel electrophoresis. The purity of each subunit preparation in a labeled (10 to 100,000 cpm) and unlabeled form was assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (24). The subunits (25 to 50 μg in 50 μl of 0.5% SDS and 0.5% 2-mercaptoethanol) were heated at 100°C for 2 min, and, after the addition of 20 μl of a solution containing 25% sucrose, 0.1% 2-mercaptoethanol, and 0.05% bromophenol blue, the samples were layered onto gels (4 by 60 mm). Polymerized gels contained 8% acrylamide, 0.3% methylenebisacrylamide, 0.002% N,N,N',N' -tetramethylethylenediamine, 0.8 mg of ammonium persulfate per ml, and 0.1% SDS in 0.1 M sodium phosphate (pH 7.2). The electrophoresis buffer was 0.1 M sodium phosphate (pH 7.2) containing 0.1% SDS. Electrophoresis was carried out at 8 mA per gel until the tracking dye migrated to the end of the gel. The gels of unlabeled subunit were stained with Coomassie brilliant blue (R-250) as described by Siegest and Jackson (18). Gels of iodinated HA were frozen and sliced into 1-mm fractions, and the radioactivity in each fraction was determined. The apparent molecular weights were determined from a standard curve constructed with the following proteins: trypsin, light-chain or heavy-chain of immunoglobulin G, ovalbumin, bovine serum albumin, and β -galactosidase.

RIP and competition procedures. Serum AHAB assays were performed in siliconized glass tubes (10 by 75 mm) or disposable polystyrene culture tubes (12 by 75 mm; Sherwood Medical Industries, Inc., St. Louis, Mo.). Each reaction tube contained equal volumes (50 μl) of the following reagents: 10% fetal calf serum, phosphate-buffered saline containing 1 mg of Pentex bovine serum albumin (Miles Laboratories) per ml, 0.5% carrier serum, and a dilution of serum and iodinated HA subunit containing approximately 10,000 cpm. Each reagent was prepared in RIP buffer: phosphate-buffered saline (pH 7.2) containing 0.1 mg of bovine serum albumin per ml, 0.1 mM ethylenediaminetetraacetic acid, and 0.03% Triton X-100. Carrier serum (free of AHAB) for use with guinea pig antisera was obtained from an unimmunized animal and, for use with human sera, from an 11-month-old child (sera from eight children between the ages of 9 and 15 months were screened as potential carrier sera, and only four were found to be suitable in their lack of detectable antibody activity). The tubes were incubated at 37°C for 2 h, followed by 20 h at 4°C . Goat anti-human immunoglobulin G or goat anti-guinea pig 7S immunoglobulins (Meloy Laboratories, Springfield, Va.) in equivalence with the carrier serum was added, and the reaction mixture was incubated at 4°C for 16 to 20 h. After the last incubation step, phosphate-buffered saline (0.5 ml) was added to all tubes before centrifugation at $3,000 \times g$ for 30 min. The level of

radioactivity in both the pellets and the supernatant fluids was determined, and the highest dilution of an antiserum that precipitated 35% of the antigen was taken as the end point. Titers are expressed as the reciprocal of this dilution. Competition assays were performed essentially as described above. Dilutions of unlabeled HA were incubated with a constant concentration of antiserum for 2 h at 37°C before the addition of labeled antigen.

Other procedures. In addition to RIP, serum AHAB titers were determined by the standard hemagglutination-inhibition (HAI) test in microtiter plates (17). These tests were performed with antigenic hybrids possessing the relevant (H3) HA and the neuraminidase of A/equine 1/Prague/56 virus. Viral neutralization (Neut) tests using rhesus monkey kidney cells were done as previously described using antigenic hybrids (32 to 150 50% tissue culture infective doses) as the test viruses (13). Titers obtained by these tests are expressed as the reciprocal of the serum dilutions.

Protein determinations were performed as described by Lowry et al. (12) and also with the fluorescamine reagent (22). Bovine serum albumin (Pentex) was the standard in both methods.

RESULTS

Characterization of the iodinated HA. Experiments to evaluate the effect of iodination procedures were performed with the A/HK/68 HA. Preparations of varying specific activity (1.9 to 23 $\mu\text{Ci}/\mu\text{g}$) were obtained by altering both the protein concentration and the reaction time. Preparations labeled to a specific activity of up to 10 $\mu\text{Ci}/\mu\text{g}$ were greater than 95% precipitable by trichloroacetic acid, and greater than 80% of the radioactivity was precipitable by specific guinea pig antiserum. Iodinated HA with a specific activity of greater than 12 $\mu\text{Ci}/\mu\text{g}$ showed reduced reactivity with specific antiserum and therefore was unsuitable for use in the RIP assay.

Stability of the labeled product during storage at -70°C was assessed at selected time intervals (Table 1). A gradual decrease in precipitability by both trichloroacetic acid and antibody was observed. To ensure that antigen used in each RIP was of comparable quality, labeled antigens were rechromatographed on a 25-ml column of Sepharose 6B just before use. The column was equilibrated and developed in phosphate-buffered saline containing 0.1% SDS and 0.1 mg of bovine serum albumin per ml. By incorporating the "cleanup" column into the routine procedure, the preparations could be used for up to 30 days.

Disc gel electrophoresis in the presence of SDS and 2-mercaptoethanol as a reducing agent established the purity of the iodinated antigens. The pattern obtained for the A/HK/68 HA is shown in Fig. 1. Two peaks containing 95% of the radioactivity were found, which correspond

TABLE 1. *Effect of storage on the precipitability of ^{125}I -labeled A/HK/68 HA^a*

Days after labeling	Maximum precipitation with antibody ^b (% of total cpm)	Maximum precipitation with TCA ^c (% of total cpm)
3	88	96
11	86	92
14	76	82
20	75	83
24	74	83
45	66	77

^a Specific activity of this preparation was 7.3 $\mu\text{Ci}/\mu\text{g}$, and the antigen was stored in 25% fetal calf serum at -70°C .

^b Guinea pig antiserum raised to the purified HA of A/HK/68 was used as the source of antibody. This antiserum had an HAI titer of 1:10,240 and was used at a dilution of 1:200.

^c TCA, Trichloroacetic acid; final concentration, 10%.

FIG. 1. *SDS-polyacrylamide gel electrophoresis of ^{125}I -labeled A/HK/68 HA. A sample of the radiolabeled HA was dissolved in 1% SDS containing 1% β -mercaptoethanol and subjected to electrophoresis on an 8% polyacrylamide gel containing 0.1% SDS at 8 mA per gel. Migration through the gel was from right to left. The gel was sliced (1-mm fractions) and counted for ^{125}I activity.*

to the two nonidentical polypeptide chains of the HA. The peak at fraction 22 was identified as HA₁, and the smaller peak at fraction 6 was identified as HA₂ by comparison of the relative migration rates with those observed with unlabeled HA. The remaining ^{125}I was recovered in the region of higher molecular weight and may have been due to incomplete reduction of the disulfide bonds; these bands were not observed with the native HA. Similar patterns were obtained with iodinated preparations of A/Eng/72 and A/PC/73.

By comparison of the relative migration rates with those observed for proteins of known molecular weights, apparent molecular weights of

48,000 and 21,000 were calculated for the HA₁ and HA₂, respectively. These values are in good agreement with those reported by others (9, 19, 20, 23).

Incubation conditions. Optimal precipitation conditions were determined by varying both the incubation time and temperature using the iodinated HA of A/HK/68 and a dilution of homologous guinea pig antiserum that precipitated less than the maximal amount of antigen (Table 2). Under the conditions evaluated, the primary reaction of labeled HA with antibody was observed to be maximal after 2 h at 37°C followed by 20 h at 4°C , and the second antigen antibody reaction was completely precipitated after 18 h at 4°C . These reactions were not affected by changes in pH between the values of 6.5 and 7.5, concentrations of NaCl between 100 and 200 mM, or concentrations of Triton X-100 between 0 and 0.3% (data not shown). Similar results were obtained with human sera.

Typical antibody titration curves are shown in Fig. 2. Iodinated preparations of purified A/HK/68 and A/Eng/72 HA and the incubation conditions determined above were used to determine the antibody titers of anti-A/HK/68 guinea pig antiserum. No antibody to either HA was detected in preimmunization serum. Using 35% precipitation, which falls on the linear portion of the curve (70 to 20%), as the end point, the titers were 38,400 and 1,500,000 for A/Eng/72 and A/HK/68, respectively. The HAI antibody titers for this antiserum were 640 and 10,240 for these two variants, respectively. The integrity of each antigen was indicated by the capacity of the antiserum to precipitate 90% of the homologous HA and 92% of the heterologous HA.

To evaluate the reproducibility of the RIP assay, nine replicate tests were performed over

TABLE 2. *Effect of time and temperature on RIP sensitivity*

Conditions during reaction 1		Conditions during reaction 2		Precipitation with antibody ^a (% of total cpm)
Hours at 37°C	Hours at 4°C	Hours at 37°C	Hours at 4°C	
0	20	0	18	25.0
1	20	0	18	34.6
2	20	0	18	38.3
4	20	0	18	40.5
2	20	0	18	43.6
2	20	2	18	39.6
2	20	4	18	41.1

^a ^{125}I -labeled A/HK/68 HA (specific activity of 3.7 $\mu\text{Ci}/\mu\text{g}$) was used as the test antigen.

a 4-day period with this antiserum and iodinated A/Eng/72 HA as the test antigen. The titers ranged from 38,400 to 48,100 with an arithmetic mean of 44,130 and a standard error of 3,815 ($\pm 9\%$).

Comparison of the RIP assay and conventional methods for determining serum AHAb titers. Human serum specimens obtained before and after a natural infection with

A/Eng/72 were available to us from a previous investigation (6). These sera were used to compare the relative sensitivity of the RIP assay to other serological methods for determining serum AHAb titers (Table 3). Three individuals by Neut, two by HAI, and all nine individuals by RIP assay had preexisting antibody to the challenge virus. The geometric mean titers were 5.8, 7.1, and 1,671 as determined by the three tests.

A fourfold or greater rise in titer is generally considered to be significant for HAI and Neut tests. From the standard error ($\pm 9\%$) determined by replicate tests on guinea pig antiserum, a twofold rise in RIP antibody titer was considered to be significant. Based on these criteria, all nine individuals experienced such rises in serum antibody titers by all three methods following infection. The geometric mean titers of the post-infection sera were 118, 161, and 18,822 for HAI, Neut, and RIP tests, respectively. Although the titers determined by RIP were higher in all cases than the other two procedures, the relative increase was not consistent. For example, the RIP titer for individual C was 23- and 73-fold higher than those of Neut and HAI, respectively, whereas the same ratios were 351 and 1,125 for individual F. The absence of a constant ratio between the AHAb titers determined by RIP and the other two procedures suggested either that the RIP assay was detecting a population of antibodies that do not inhibit viral hemagglutination and/or infectivity, or that the antisera differed in their relative avidity for the A/Eng/72 HA, or a combination of both factors. These possibilities could arise in humans through previous exposures to other strains of influenza viruses.

To determine whether the variability in titer ratios observed with human specimens was in-

FIG. 2. Titration of guinea pig anti-A/HK/68 antiserum by RIP tests. Serial dilutions of antiserum to native A/HK/68 HA were reacted with ^{125}I -labeled A/Eng/72 HA (Δ) and ^{125}I -labeled A/HK/68 HA ($\bullet$) in double antibody RIP assays. The specific activities for the test antigens were $9.8 \mu\text{Ci}/\mu\text{g}$ for the A/Eng/72 and $7.3 \mu\text{Ci}/\mu\text{g}$ for the A/HK/68.

TABLE 3. Comparison of human serum AHAb titers obtained by RIP and conventional methods before and after natural infection with A/Eng/72 (H3N2) influenza virus^a

Individual	Serum AHAb titers ^b					
	Preinfection			Postinfection		
	HAI	Neut	RIP	HAI	Neut	RIP
A	<10	<4	1,600	160	256	52,000
B	<10	<4	820	80	256	16,000
C	<10	<4	950	80	256	5,800
D	<10	<4	1,700	80	32	6,200
E	<10	<4	1,450	80	64	6,300
F	<10	<4	2,400	160	512	180,000
G	10	16	4,000	160	256	18,500
H	<10	16	2,150	80	64	6,300
I	10	16	1,600	320	256	75,000

^a Infection was documented in all individuals by viral isolation in addition to a serological rise.

^b Titers are expressed as the reciprocal of the serum dilution. HAI and Neut tests were performed with an antigenic hybrid possessing the HA of A/Eng/72 and the neuraminidase of equine 1. RIP assays were carried out using ^{125}I -labeled A/Eng/72 HA (specific activity $9.8 \mu\text{Ci}/\mu\text{g}$) as the test antigen.

fluenced by preexisting antibody, guinea pig antisera were evaluated by the three methods (Table 4). Since no antibody to the three variants was detected by RIP in any of the preimmunization sera, only postimmunization antibody titers are presented. Although there was a general correlation between the antibody titers determined by the three procedures, again no consistent ratio was observed between the AHAb titers determined by RIP and the other two procedures. For example, the homologous antibody titers determined by RIP ranged from 2.5- to 188-fold higher than HAI, and the average increase was 85-fold. The increased sensitivity of the RIP assay was more pronounced in comparisons of the AHAb responses to heterologous HA. In 13 comparisons, the RIP titers ranged from 5- to 1,025-fold higher than HAI, and the average increase was 225-fold. The same RIP titers were 5 to 1,640 times higher than the corresponding Neut titers, and the average increase was 317. The latter results suggest that cross-reactive antibodies detectable by the RIP procedure may be less efficient at inhibiting hemagglutination and infectivity than is "variant specific" antibody.

Competition assays. Experiments performed with unlabeled purified A/HK/68 HA subunits to determine the sensitivity of the RIP assay for the detection of antigen by using two dilutions of guinea pig antiserum are presented in Fig. 3. A 1:100,000 dilution of this antiserum precipitated 85% of the radioactivity; this represented an antibody concentration substantially higher than that which fell on the linear portion of the precipitation curve (see Fig. 2). A large amount of competing antigen, 200 ng, was required to inhibit this reaction by 50%. On the other hand, a 1:800,000 antiserum dilution precipitated 48% of the radioactivity in the absence of competing antigen, and 1 ng of unlabeled HA was sufficient to effect a 50% inhibition; as little as 0.5 ng was sufficient to inhibit the reaction by 30%.

DISCUSSION

The sensitivity of any RIP assay is dependent upon the specific activity of the labeled antigen (2). The data presented in this report demonstrated that the purified intact HA subunits can be radiolabeled to a high specific activity by the chloramine T procedure without any detectable loss of antigenicity. Although both the HA₁ and HA₂ polypeptides were iodinated, the majority (>70%) of the label was associated with the HA₁. Russ et al. (14) found a similar distribution for the HA subunits of an A/PC/73-like strain after iodination of the bromelain-solubilized products. However, the location of the radioactive iodine may vary for HA of different type A variants. In this regard, studies utilizing either purified

FIG. 3. Competitive inhibition RIP using ¹²⁵I-labeled A/HK/68 HA and increasing concentrations of unlabeled A/HK/68 HA. The A/HK/68 antiserum used was the same as that shown in Fig. 2, and the appropriate dilution was determined from those data. The curve to the left (○) was generated using a 1:800,000 dilution of this antiserum, which precipitated 48% of the radioactivity in the absence of competing antigen, and the curve to the right (●) was generated using a 1:100,000 dilution, which was capable of precipitating 85% of the radioactivity.

TABLE 4. Guinea pig AHAb titers obtained by conventional methods and RIP^a

Guinea pig no.	Immunizing antigen	HAI			Neut			RIP		
		HK	Eng	PC	HK	Eng	PC	HK	Eng	PC
1	HK HA	10,240	640	160	64,000	256	100	1,500,000	44,000	25,000
2	HK HA	20,480	640	160	128,000	512	100	3,000,000	340,000	70,000
3	Eng HA	80	640	640	256	ND	1,000	ND	120,000	102,000
4	Eng HA	40	2,560	1,280	25	ND	6,400	41,000	83,000	21,000
5	Eng WV	640	2,560	2,560	1,600	ND	6,400	ND	152,000	70,000
6	PC HA	320	640	1,280	1,000	1,024	12,800	ND	31,000	27,000
7	PC WV	640	6,400	40,960	1,600	ND	16,000	ND	51,000	100,000
8	Scot HA	1,280	640	10,240	3,200	1,024	10,000	300,000	140,000	51,000

^a RIP procedures were carried out using iodinated preparations of the indicated HA; specific activities were 7.3, 10.9, and 9.8 for A/HK/68 (HK), A/Eng/72 (Eng), and A/PC/73 (PC), respectively. Scot, A/Scotland/74 virus; ND, not done; WV, whole virus.

whole virus or solubilized proteins of A/Bel/42 (HON1) have shown that the HA₂ of this HA is more heavily iodinated (21). This variability would be expected, since differences in the primary amino acid sequence of both polypeptide chains shown by peptide mapping of HA of different subtypes has been reported (11).

We attempted to compare the sensitivity of the RIP technique for the detection of antibody with that of RIA results previously published by others (3-5, 10, 14, 15). Since the RIP assay described in this report measures "total" antigen binding capacity, a minimum estimate of the specific AHAb antibody required for antigen precipitation was computed. Based on the specific activity and quantity of ¹²⁵I in the reaction mixture, each assay tube contained approximately 0.8 ng of antigen. By this computation, 35% precipitation represented 0.28 ng; this estimate was supported by the competition data, where 0.5 ng of unlabeled HA inhibited the binding by 30%. Assuming the HA to be a trimer of molecular weight 240,000 under the conditions of the assay and assuming the antigen binding sites to be saturated, 0.1 ng of immunoglobulin G would be required to precipitate 35% of the labeled HA. Estimates of the minimum specific serum AHAb levels detectable by solid-phase RIA have ranged from 0.25 (5) to 37 ng (4). This suggests that the RIP assay is comparable in sensitivity to solid-phase RIA and, in addition, offers the distinct advantage of utilizing purified HA as the test antigens.

The human sera chosen for study were from a previous study in which infection with an A/Eng/72 (H3N2)-like virus had been documented for each individual by virus isolation in addition to a serological rise by standard methods (6). A surprising aspect of these experiments was the level of antibody present prior to infection. Cross-reactivity between the A/Eng/72 variant and its immediate predecessor in human populations, A/HK/68, has been well established. Therefore it was expected that some individuals would have low antibody levels to the challenge virus at the time of infection. However, the high frequency and level of AHAb shown by the RIP assay were unexpected. Several lines of evidence support the conclusion that these relatively high RIP titers were in fact due to antibody. Pretreatment of the sera with neuraminidase to destroy inhibitors of hemagglutination did not change the titers; four of eight sera taken from children between the ages of 9 and 15 months, with no history of an influenza-like illness, were free of detectable antibody in RIP assays to the H3 HA; all of the guinea pigs were antibody negative before immunization; and after infection all nine individuals experienced a

significant increase in serum AHAb by RIP assay. Although the prechallenge antibody may simply reflect a previous exposure to the A/HK/68 variant, it seems possible that the RIP assay is sensitive to cross-reacting antibodies produced to HA of previous subtypes.

Comparisons of antibody titers on acute- and convalescent-phase sera from persons infected with A/Eng/72 revealed significant postinfection elevations by RIP, HAI, and Neut tests. A generally positive correlation existed between the antibody titers determined by these three procedures (i.e., sera with the higher RIP titers tended to have higher titers by HAI and Neut), but the AHAb levels determined by RIP could not be related to those by HAI and Neut by a constant factor. These comparisons were complicated by the presence of preinfection cross-reactive antibody of unknown origin. Accordingly, a further comparison of antibody titers measured by the three procedures was carried out using hyperimmune guinea pig antisera prepared against single variants. Despite the fact that the animals were immunized with the same antigen dose and were bled after the same interval, ratios between AHAb titers determined by the RIP assay and those determined by HAI and Neut were highly variable. Since RIP measures the total antigen binding capacity of an antiserum, the absence of a consistent ratio suggests either that antibodies produced to certain antigenic determinants do not inhibit hemagglutination or neutralize virus or that the antisera may vary considerably in their affinity for the different HA. The present data do not distinguish between these two possibilities. However, competition assays utilizing both purified homologous or heterologous HA as the competing antigens should more clearly define the immunochemical basis for the cross-reactivity. Experiments to determine the applicability of this methodology for determining the antigenic relationships of the HA of influenza viruses are currently being performed.

The sensitivity of the competition assay for detection of antigen was found to be comparable to that reported for other viral antigens (see 16, and the references cited therein). This aspect of the RIP methodology may be applicable to other areas of influenza research, such as investigations dealing with the occurrence of viremia and antigenemia with influenza virus infections.

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