APPLIED MICROBIOLOGY, Feb. 1971, p. 217-226
Vol. 21, No. 2

Comparison of Various Methods for Preparation of Viral Serological Antigens from Infected Cell Cultures

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Received for publication 20 July 1970

In efforts to prepare more potent and sensitive viral serological antigens, several aspects of the production of antigens from infected cell cultures were studied. Antigens derived from whole, infected culture material and from the cellular and fluid phases were compared. Freezing and thawing, sonication, and alkaline buffer extraction were compared for effectiveness in releasing antigen from host cells. The effect of the multiplicity of infection on titers of viral antigens produced in cell cultures was studied. Generally, higher titered antigens were derived from the infected cells than from the culture fluids, but for certain viruses complement-fixing (CF) antigens derived from the culture fluids gave higher antibody titers than did cell-associated antigens. With each virus-host cell system studied, treatment with alkaline buffers extracted appreciable amounts of CF antigen from the host cells, but in some instances more antigen was released by freezing and thawing or by sonication. Extraction of infected cells with alkaline buffers was not a satisfactory method for preparation of hemagglutinating (HA) antigens for any of the viruses studied. The highest-titered HA antigens were produced from infected cells disrupted by freezing and thawing or sonication. The highest titered CF and HA antigens were produced from cell cultures infected at multiplicities of one or greater. Complement-fixing antigens produced by infecting cells in suspension and then planting had lower titers than antigens produced in parallel by infecting developed monolayers. Optimal methods are summarized for preparation of serological antigens to a variety of viruses of man.

One of the essential needs of viral diagnostic laboratories is the ready availability of serological antigens of high potency and specificity for use in complement fixation (CF) or hemagglutination inhibition (HI) tests. In addition to adequate potency, CF antigens must be free from anticomplementary (AC) activity at dilutions containing 2 or 4 antigenic units. Viral hemagglutinating (HA) antigens must be free from serum, host tissue, or microbial contaminants which might agglutinate the test erythrocytes; they must also be free from nonspecific inhibitors of viral hemagglutinins.

In recent years several methods have been described for increasing the titer of viral serological antigens produced in cell cultures. These include the infection of large numbers of host cells in a small volume of medium (3, 23, 27), preparation of antigens from the concentrated cellular phase of infected cultures (1, 17, 25), extraction of antigen from host cells by treatment with alkaline buffers (7, 8, 22), and infection of host cells in suspension and then growing them into monolayer cultures (24, 26).

It is well recognized that no single method of preparation gives satisfactory titers for all viral antigens, and optimal conditions for antigen production must be individually defined for each group of viruses and type of antigen. In the present investigation methods were explored for producing higher titered antigens from representative viruses of the major groups which produce infection in man. Several aspects of viral CF and HA antigen production were investigated. A comparison was made of the potency and sensitivity of antigens derived from the fluid and from the cellular phases of infected cell cultures, and various methods were compared for effectiveness in releasing viral antigens from host cells. A study was made of the effect of the multiplicity of infection on the potency of viral CF and HA antigens produced in cell cultures. Also,

a comparison was made of the potency of CF antigens produced in parallel by infecting host cells in suspension and by infecting monolayer cultures.

MATERIALS AND METHODS

Cell cultures. The following cell culture systems were used for production of viral antigens: HeLa cells of the D line, KB cells (4), the BHK-21 line of baby hamster kidney cells (13), the Vero line of grivet monkey kidney cells (28), a human fetal diploid lung line developed by J. H. Schieble of this laboratory, primary or secondary rhesus monkey kidney cells, and primary chick embryo tissue cultures (CETC). Cells were propagated by methods which have been described elsewhere (19).

Serological tests. Antigens were assayed for CF activity by the standard microtechnique of this laboratory (11); they were examined in block titrations, and the antigen titers were expressed in terms of the highest dilution of antigen giving 3+ or 4+ CF with the highest dilution of immune serum. HA tests were also performed by the micromethod; conditions used for HA tests with the different viruses are described elsewhere (21).

Infectivity titrations. Infectivity titers of the seed virus preparations employed for antigen production were determined by various methods. Respiratory syncytial (RS) virus (18) and reovirus type 3 (12) were assayed by fluorescent cell counting. Arboviruses (5) and adenoviruses (16) were assayed by plaquing. Myxoviruses were titrated in tube cultures using hemadsorption as an end point; vaccinia, cytomegalovirus, and echovirus type 6 were titrated in tube cultures using a cytopathic effect as an end point (19). The virus strains employed are indicated in the tables.

Preparation of antigens from the cellular and fluid phases of infected cultures. Cells of the appropriate type were cultured in 8-oz prescription bottles, and, when they had grown into confluent monolayers, the outgrowth medium was changed to maintenance medium; this consisted of 5% inactivated fetal bovine serum and 95% Leibovitz medium no. 15 (L-15) (5) for measles antigen, and of 2% fetal bovine serum and 98% L-15 for the other viruses. Cultures were then inoculated with virus. The ratio of viral infectious doses per cell (multiplicity of infection) employed for each system is given in Tables 1 and 2. Infected cultures were incubated at 36 C and harvested when they showed a 4+ viral cytopathic effect (CPE). The cells and fluids from three replicate cultures were pooled, and a sample of the whole culture material was removed and subjected to sonic oscillation at 20 kc/sec for 2 min. The remainder of the culture material was divided into three equal portions which were centrifuged at 600 × *g* for 20 min. The supernatant fluids were removed and pooled. Two samples of the cells were each resuspended in Hanks balanced salt solution (BSS) to one-tenth of the original volume; one of these was frozen and thawed three times and the other was sonically treated. The third sample of cells was extracted in 0.1 M glycine buffer, *p*H 9.5 (one-tenth of the original volume), at 37 C for 6 hr. The material was then centrifuged at 600 × *g* for 20 min, and the supernatant fluid (extract) was collected. The extracted cells were suspended in one-tenth of the original volume of Hanks BSS and sonically treated.

Thus, antigens were prepared in parallel from (i) the whole culture, (ii) the fluid phase, (iii) the cellular phase, 10×, disrupted by freezing and thawing; (iv) the cellular phase, 10×, disrupted by sonic oscillation; (v) a glycine buffer extract of cells, 10×; and (vi) the cells after alkaline buffer extraction, 10×. Control antigens were prepared in the same manner from uninfected cell cultures.

Preparation of antigens using various multiplicities of infection. Cell cultures of the appropriate type were prepared in 8-oz culture bottles. When the cells had grown into confluent monolayers, the growth medium was removed and duplicate cultures were infected with 10, 1, and 0.1 infectious doses of virus per cell. Each concentration of virus was suspended in the same volume of fluid. After incubation at 36 C for 90 min the inocula were removed, and 10 ml of maintenance medium, consisting of 2% inactivated fetal bovine serum and 98% L-15, was added to each culture. Cultures were incubated at 36 C and harvested when they showed a 4+ viral CPE. The cellular and fluid phases of each culture were separated by centrifugation at 600 × *g* for 20 min. After removal of the fluid phase, the cells were resuspended in Hanks BSS to one-tenth of the original culture volume and sonicated at 20 kc/sec for 2 min. Control antigens were prepared in the same manner from uninfected cell cultures.

Preparation of antigens in parallel by infecting cells in suspension and in monolayers. Cells of the appropriate type were grown to confluency in 8-oz culture bottles. Cell counts were made on representative cultures, and cells were then infected at a ratio of 1 to 10 infectious doses of virus per cell by using two procedures in parallel. In the first, the inoculum was added to monolayer cultures, and after an incubation period of 90 min at 36 C the inoculum was removed and growth medium was added to the cultures. In the second, trypsin-dispersed cells were suspended in the viral inoculum and held at 36 C for 90 min with occasional shaking. The inoculum was then removed, and the infected cells were suspended in growth medium and planted in 8-oz culture bottles to give the same number of cells as that contained in the corresponding monolayer cultures. After incubation at 36 C for 18 to 24 hr, the growth medium was removed from both sets of cultures and replaced with serum-free Eagle's minimal essential medium. Cultures from each set were harvested separately after 3 additional days of incubation, and the cellular and fluid phases were separated by centrifugation at 600 × *g* for 20 min. After removal of the fluid phase, the cells were suspended in Hanks BSS to one-tenth of the original culture volume and then subjected to sonic oscillation at 20 kc/sec for 2 min. Controls for each set of infected cultures were prepared in the same manner from uninfected cells.

TABLE 1. *Comparison of the potency and sensitivity of viral complement fixation (CF) antigens derived from whole cell culture material and from the fluid and cellular phases*

Virus	Strain	Host cell culture system[a]	MOI[b]	Day of harvest	CF titers of					
					Whole culture	Culture fluid	Cells 10× F and T[c] 3×	Cells 10×, sonically treated	Cells 10×, alkaline extracted	
									Extract	Cells after extraction
Respiratory syncytial	Balin	HeLa	3	6	8[d] (256)[e]	4 (256)	32 (64)	16 (64)	8 (128)	8 (128)
Measles	Edmonston	HeLa	1	5	16 (256)	8 (128)	64 (256)	64 (256)	32 (256)	16 (128)
Parainfluenza type 2	Greer	MK	6	3	4 (128)	4 (128)	8 (256)	16 (256)	4 (256)	2 (256)
St. Louis encephalitis	Ruis	Vero	3	5	2 (8)	2 (8)	4 (32)	4 (32)	2 (64)	<2
St. Louis encephalitis	Ruis	BHK-21	3	5	<2	<2	2(64)	4 (64)	2 (32)	2 (8)
Colorado tick fever	Florio	Vero	9	2	4 (64)	4 (32)	32 (32)	16 (32)	8 (64)	<2
Colorado tick fever	Florio	BHK-21	9	2	8 (32)	4 (32)	64 (32)	64 (32)	64 (32)	4 (32)
Western equine encephalitis	B628 clone 15	Vero	10	3	2 (32)	2 (32)	4 (32)	4 (32)	4 (128)	<2
	B628 clone 15	BHK-21	10	2	4 (64)	4 (64)	4 (128)	4 (128)	4 (128)	2 (8)
Herpes simplex	Mc Intyre	CETC	5	2	4 (32)	2 (32)	8 (32)	16 (32)	4 (32)	4 (32)
		BHK-21	9	2	4 (64)	2 (64)	16 (32)	16 (64)	8 (64)	2 (16)
Cytomegalovirus	AD-169	HFDL	0.01	6	8 (64)	4 (64)	32 (64)	32 (32)	8 (64)	8 (64)
Vaccinia	Butler	CETC	1	3	32[f] (64)	16[f] (64)	32[f] (32)	32[f] (32)	32 (64)	8 (64)
Vaccinia	Butler	BHK-21	3	3	32[f] (64)	16[f] (64)	32[f] (32)	32[f] (32)	32 (64)	4 (32)
Adenovirus type 14	DeWit	HeLa	0.004	4	16 (64)	8 (64)	64 (16)	64 (32)	≥64 (32)	16 (64)
Adenovirus type 28	BP-5	HeLa	0.004	3	32 (128)	8 (128)	512 (64)	512 (64)	512 (64)	128 (64)

[a] Abbreviations: MK, monkey kidney; BHK, baby hamster kidney; CETC, chick embryo tissue culture; HFDL, human fetal diploid lung. [b] Multiplicity of infection. [c] Frozen and thawed. [d] Reciprocal of antigen titer. [e] Reciprocal of antibody titer in parentheses. [f] Antigen anticomplementary at dilutions containing 2 or 4 units.

RESULTS

Comparison of antigens prepared from the cellular and fluid phases of infected cultures and a comparison of various methods for releasing cell-associated antigen. In defining the optimal conditions for production of serological antigens, it must be determined whether the antigen is released readily into the culture fluid or whether it remains largely cell associated. In instances in which several distinct viral antigens are produced in a cell culture system, some of the antigens may be released more rapidly or completely than others. Viral antigens are generally dissociated from host cells by freezing and thawing or by sonic oscillation, but it has been shown that extraction of rubella virus-infected cells with alkaline buffers is a more effective method than freezing and thawing or sonication for releasing CF antigen (22). The possibility was considered that alkaline extraction might be effective in producing more potent serological antigens for certain other viruses.

Table 1 compares the CF titers of antigens produced in parallel from whole culture material, culture fluids, cells disrupted by freezing and thawing, by sonic treatment, and by alkaline buffer extraction of the cells. In addition to potency, an equally important attribute of a CF antigen is its sensitivity for demonstrating antibody; some antigens with high titers may give relatively low antibody end points. Table 1 shows both the CF antigen and antibody titers obtained with each preparation.

HA titers of some of the antigen preparations are shown in Table 2.

With RS virus, the highest CF antigen titers were obtained from infected cells concentrated 10× and disrupted by freezing and thawing or by sonication. However, antigens derived from the whole culture material or from the culture fluids gave the highest antibody titers, suggesting that certain RS antigens are rapidly released into the culture fluids. Some CF antigen was demonstrable in the alkaline buffer extract and in the cells after extraction.

The most potent CF and HA antigens for measles and parainfluenza type 2 viruses were produced from the cells concentrated 10× and

TABLE 2. *Comparison of the hemagglutinating (HA) titers of viral antigens derived from whole cell culture material and from the fluid and cellular phases*

Virus	Strain	Host cell culture system[a]	MOI[b]	Day of harvest	HA titers of					
					Whole culture	Culture fluid	Cells 10× F and T[c] 3×	Cells 10× sonically treated	Cells 10× alkaline extracted	
									Extract	Cells after extraction
Measles	Edmonston	HeLa	1	5	64[d]	16	256	256	16	64
Parainfluenza type 2	Greer	MK	6	3	16	<2	64	16	<2	2
Vaccinia	Butler	CETC	1	3	8	8	128	64	<2	<2
Vaccinia	Butler	BHK-21	3	3	4	4	16	32	4	<2
Adenovirus type 14	DeWit	HeLa	0.004	4	4	<2	256	128	4	2
Adenovirus type 28	BP-5	HeLa	0.004	3	4	4	8	8	<2	4
Reovirus type 3	Abney	BHK-21	2	2	<2	<2	512	512	<2	<2
Reovirus type 3	Abney	BHK-21	0.5	6	64	32	128	256	<2	8

[a] Abbreviations: MK, monkey kidney; CETC, chick embryo tissue culture; BHK, baby hamster kidney.
[b] Multiplicity of infection.
[c] Frozen and thawed.
[d] Reciprocal of hemagglutination titer.

disrupted by freezing and thawing or by sonication. CF antigen was also demonstrable in the alkaline extract and in the cells after extraction, but little or no HA activity was demonstrable in these fractions.

Previous attempts in this laboratory to produce CF antigens for St. Louis encephalitis (SLE) in cell cultures were unsuccessful, and in the experiments shown in Table 1 only low levels of antigen were demonstrable in the various fractions. The most satisfactory antigen from the standpoint of potency and specificity was produced from infected BHK-21 cells concentrated 10× and sonically treated. Using the same procedure but concentrating the cells 50×, CF antigens with titers as high as 1:32 have subsequently been produced. These cell culture-derived SLE antigens give slightly higher CF antibody titers than do antigens prepared from mouse brain tissue.

The most potent and sensitive CF antigens for Colorado tick fever were derived from infected BHK-21 cells concentrated 10×. Equally potent antigens were produced by freezing and thawing, by sonication, or by alkaline buffer extraction of the cells, but alkaline buffer extracts were less cloudy and more homogenous than antigens prepared by the other two methods.

CF antigens for Western equine encephalitis (WEE) have been routinely produced in this laboratory using CETC infected with the 46-A42 virus strain. However, it was considered desirable to produce antigens from the attenuated clone 15 of the B628 strain (9) if possible. Antigens with CF titers of 1:4 were derived from both the cellular and fluid phases of infected BHK-21 cultures. Alkaline buffer extracts were the most satisfactory antigens from the standpoint of sensitivity and lack of AC activity. By extracting infected BHK-21 cells in alkaline buffer at 0.05 or 0.02 of the original culture volume, antigens titering 1:16 have been produced from the attenuated WEE virus strain.

The most potent and sensitive CF antigens for herpes simplex virus were prepared from infected cells concentrated 10× and disrupted by sonication. The most satisfactory CF antigen for cytomegalovirus (CMV) was derived from cells concentrated 10× and disrupted by freezing and thawing. Lower levels of herpes simplex and CMV antigen were extracted from the cells with alkaline buffer, but these extracts gave antibody titers comparable to those obtained with the other antigens.

Vaccinia CF antigens produced from whole infected culture material, from culture fluids, or from cells disrupted by freezing and thawing or by sonication were all AC at dilutions containing 2 or 4 antigenic units. However, alkaline buffer extraction of infected CETC or BHK-21 cells yielded high-titered and sensitive CF antigens which were free from AC activity. As shown in Table 2, satisfactory HA antigens for vaccinia were derived from the infected cells only by freezing and thawing or by sonication but not by alkaline buffer extraction.

Adenovirus CF antigens produced from infected host cells by freezing and thawing, by sonic treatment, or by alkaline buffer extraction all had high titers, but they gave antibody end points slightly lower than did antigens produced from the whole culture or from culture fluids. Appreciable CF activity was present in adenovirus-infected cells after alkaline extraction. Satisfactory HA antigens for adenovirus types 14 and 28 were produced only from infected cells concentrated 10× and disrupted by freezing and thawing or by sonication.

In BHK-21 cultures infected with reovirus type 3 at a high multiplicity and harvested as soon as CPE was complete, HA antigen was demonstrable only in the infected cells concentrated 10× and disrupted by freezing and thawing or by sonication. In cultures infected at a lower multiplicity and harvested later, HA antigen was also demonstrable in the culture fluid. Hemagglutinating antigen was not demonstrable in alkaline extracts of the cells.

Studies were conducted on certain antigens prepared by freezing and thawing or by sonication of infected cells to determine the effect of clarification by low-speed centrifugation. It has previously been shown in this laboratory that clarification of varicella-zoster antigens produced by sonication of infected cells results in a loss of 75 to 90% of the CF activity. Clarification at 600 × g resulted in the loss of 50% of the CF activity of measles antigen prepared by sonication and of CMV antigen prepared by freezing and thawing infected cells, and it reduced by 50 to 75% the CF titer of herpes simplex antigen produced by sonication of infected cells.

Comparison of antigens prepared by infecting cell cultures with varying doses of virus. Table 3 shows the CF and HA titers of several viral antigens produced by infecting host cell cultures with various concentrations of virus (10, 1, and 0.1 infectious doses per cell). Cultures were harvested when the cells showed a 4+ viral CPE. In order to determine the location of the antigen at the time of harvest, the culture fluids and cells were separated for assay. The cellular phase was resuspended in Hanks BSS to one-tenth of the original culture volume and sonicated.

Titers of the herpes simplex CF antigens prepared with the three different concentrations of

TABLE 3. *Comparison of complement fixation (CF) and hemagglutinating (HA) antigens prepared by infecting cell cultures with various concentrations of virus*

Virus	Strain	Host cell culture system[a]	MOI[b]	Day of harvest	Type of antigen	Titer of antigen derived from	
						Fluid phase	Cells 10×, sonically treated
Herpes simplex	McIntyre	BHK-21	10	1	CF	2[c] (16)[d]	32 (32)
			1	2	CF	2 (16)	32 (32)
			0.1	3	CF	2 (16)	32 (32)
Respiratory syncytial	Balin	HeLa	10	6	CF	4 (256)	32 (64)
			1	7	CF	8 (512)	8 (64)
			0.1	8	CF	<2	2 (128)
Adenovirus type 3	G. B.	KB	10	2	CF	8 (128)	1,024 (64)
			1	4	CF	16 (128)	512 (64)
			0.1	5	CF	<8	64 (64)
Measles	Edmonston	HeLa	10	6	CF	16 (256)	64 (256)
			1	7	CF	16 (256)	64 (256)
			0.1	8	CF	8 (256)	32 (256)
Adenovirus type 3	G. B.	KB	10	2	HA	2	1,024
			1	4	HA	4	128
			0.1	5	HA	<2	32
Measles	Edmonston	HeLa	10	6	HA	8	128
			1	7	HA	8	128
			0.1	8	HA	8	64
Echovirus 6	Greer	MK	10	2	HA	128	1,024
			1	3	HA	32	1,024
			0.1	4	HA	32	512
Reovirus type 3	Abney	BHK-21	10	5	HA	<2	512
			1	6	HA	16	128
			0.1	7	HA	64	64

[a] Abbreviations: BHK, baby hamster kidney; MK, monkey kidney.
[b] Multiplicity of infection.
[c] Reciprocal of antigen titer.
[d] Reciprocal of antibody titer in parentheses.

virus were identical. In all instances greater amounts of antigen were present in the cells than in the fluids, and antibody end points obtained with the cell-associated antigens were slightly higher than those obtained with the infected culture fluids.

Cell cultures infected with RS virus at a multiplicity of ten yielded the highest titered CF antigens. Antigens derived from the fluid phase of cultures infected at multiplicities of one and ten gave the highest antibody titers. Little antigen was demonstrable in cultures infected at a multiplicity of one-tenth and harvested at 8 days, despite the fact that the cultures showed an extensive viral CPE.

High titers of adenovirus type 3 CF antigen were demonstrable in the cellular phase of cultures infected at a multiplicity of 1 or 10, but infected fluids from these cultures gave slightly higher antibody titers. The highest titered adenovirus type 3 HA antigen was derived from the cellular phase of cultures infected at a multiplicity of ten. Little adenovirus CF or HA antigen was demonstrable in cultures infected at a multiplicity of one-tenth.

Measles CF and HA antigens with the highest titers were those derived from the cellular phase of cultures infected at a multiplicity of one or ten.

Cell cultures infected with echovirus type 6 at a multiplicity of ten yielded greater amounts of HA antigen in both the cellular and fluid phases than did cultures infected with lower concentrations of virus.

TABLE 4. *Comparison of the potency of viral complement fixation (CF) antigens produced by infecting monolayer cultures and by infecting cells in suspension and then planting*

Virus	Strain	Host cell culture system[a]	MOI[b]	Day of harvest	CF titer of antigens prepared by infecting cells in			
					Monolayer cultures		Suspension and then planting	
					Fluid phase	Cells 10×, sonically treated	Fluid phase	Cells 10×, sonically treated
Respiratory syncytial	Balin	BHK-21	1	4	16[c]	32	8	16
Respiratory syncytial	Balin	HFDL	>1	4	4	16	2	4
Parainfluenza type 2	Greer	MK	>1	4	4	16	4	8
St. Louis encephalitis	Hubbard	BHK-21	>1	4	4	16	4	8
Western equine encephalitis	B628, clone 15	BHK-21	2	4	2	4	<2	<2
Vaccinia	Butler	CETC	>1	4	16 (AC)[d]	32 (AC)	8	16
Vaccinia	Butler	BHK-21	>1	4	16 (AC)	32 (AC)	8	16

[a] Abbreviations: BHK, baby hamster kidney; HFDL, human fetal diploid lung; MK, monkey kidney; CETC, chick embryo tissue culture.
[b] Multiplicity of infection.
[c] Reciprocal of antigen titer.
[d] Antigen anticomplementary at dilutions containing 2 or 4 units.

Cultures infected with reovirus type 3 at a multiplicity of ten had HA antigen only in the cellular phase at the time of harvest, whereas cultures infected with lower doses of virus had increased amounts of antigen demonstrable in the fluid phase and less antigen in the cellular phase.

Comparison of the potency of viral CF antigens prepared by infecting monolayers and by infecting cells in suspension and then planting. For certain viruses which produce a delayed CPE, e.g., monkey foamy viruses (26) and rubella virus (24), it has been found that more potent CF antigens are produced by infecting cells in suspensions and then planting them rather than by infecting developed monolayers. These two methods of infecting cells were tested in parallel for production of CF antigens to certain other viruses. Results are shown in Table 4. For all of the viruses studied, antigens produced by infecting monolayer cultures had slightly higher CF titers than did antigens produced in parallel by infecting cells in suspension and then planting. Vaccinia CF antigens produced by infecting monolayers were AC at dilutions containing 2 or 4 antigenic units, but antigens produced in parallel by infecting cells in suspension and then planting were not AC.

DISCUSSION

These studies helped to develop procedures for the production of higher titered serological antigens for a variety of viruses. The procedures yielding the most satisfactory antigen for each virus under study are summarized in Table 5. None of these methods for antigen production requires complex or time-consuming concentration procedures.

In some instances the titers of antigens produced by the various methods differed only slightly from one another, but comparatively certain antigens were more potent and sensitive. Titers of CF antigens as determined by "block" titrations rarely show variation from test to test, and thus the slightly higher antigen titers are reproducible, and assume importance from the practical standpoint of how far the antigen will go, i.e., how much will become available when it is appropriately diluted for use in diagnostic tests.

Most of the viral antigen produced in cell cultures was found to be cell-associated at the time the cultures first showed an extensive viral CPE. However, using only the cellular phase was not an optimal procedure for production of all antigens. In the case of RS virus and adenoviruses, the fluid phase apparently contained

TABLE 5. *Optimal methods for preparation of certain viral serological antigens*

Virus	Type of antigen[a]	Host cell culture system[b]	MOI[c]	Cell culture material used for antigen
Respiratory syncytial	CF	HeLa	3–10	Whole culture (cells and fluids) sonically treated
Measles	CF	HeLa	1–10	Cells 10X, sonically treated or frozen and thawed
Measles	HA	HeLa	1–10	Cells 10X, sonically treated or frozen and thawed
Parainfluenza type 2	CF	MK	1–10	Cells 10X, sonically treated or frozen and thawed
Parainfluenza type 2	HA	MK	1–10	Cells 10X, sonically treated or frozen and thawed
St. Louis encephalitis	CF	BHK-21	1–10	Cells 50X, sonically treated
Colorado tick fever	CF	BHK-21	1–10	Alkaline buffer[d] extract of cells 10X
Western equine encephalitis	CF	BHK-21	1–10	Alkaline buffer extract of cells 50X
Reovirus type 3	HA	BHK-21	1–10	Cells 10X, sonically treated or frozen and thawed
Echovirus type 6	HA	MK	10	Cells 10X, sonically treated or frozen and thawed
Herpes simplex	CF	BHK-21	1–10	Cells 10X, sonically treated
Cytomegalovirus	CF	HFDL	≥0.01	Cells 10X, frozen and thawed
Adenovirus	CF	HeLa or KB	≥1	Whole culture (cells and fluids) sonically treated
Adenovirus	HA	KB	≥1	Cells 10X, sonically treated or frozen and thawed
Vaccinia	CF	BHK-21 or CETC	≥1	Alkaline buffer extract of cells 10X
Vaccinia	HA	BHK-21 or CETC	≥1	Cells 10X, sonically treated or frozen and thawed

[a] CF, complement fixation; HA, hemagglutinating.
[b] Abbreviations: MK, monkey kidney; BHK, baby hamster kidney; HFDL, human fetal diploid lung; CETC, chick embryo tissue culture.
[c] Multiplicity of infection. Cultures harvested when they show 4+ viral cytopathic effect.
[d] Glycine buffer (0.1 M, *p*H 9.5) cells extracted at 37 C for 6 hr.

additional CF antigens which gave higher antibody titers than did cell-associated CF antigens. Both of these viruses are known to produce multiple soluble antigens with distinct serological specificities (6, 14), and it is possible that certain of the antigens are rapidly released into the culture fluids.

Although satisfactory CF antigens for SLE, CTF, and WEE viruses were produced from infected cell cultures, the same infected culture materials had little or no HA activity. Similarly, Pedreira et al. (15) found that infected cell cultures yielded satisfactory CF, but not HA, antigens for a variety of arboviruses.

Of particular interest was the effect of alkaline buffer extraction on cell-associated viral CF and HA antigens. With each virus-host cell system studied, CF antigen was released from the cells by this method. In the case of the arboviruses, virtually all of the CF antigen was extracted from the cells, but with the other viruses an appreciable amount of CF antigen was still demonstrable in the cells after extraction. Alkaline buffer extraction was found to be the optimal method for production of CF antigens for certain arboviruses and vaccinia; the antigens were high-titered, sensitive for detection of antibody, and free from AC activity. In the case of vaccinia, the alkaline extracts were the only preparations from either CETC or BHK-21 cells which were free

from AC activity. Another advantage of alkaline extracted CF antigens was the fact that they were more homogenous than antigens produced by disruption of cells by freezing and thawing or by sonication.

Alkaline buffer extraction failed to produce satisfactory HA antigens for any of the viruses studied, despite the fact that this procedure yields high-titered rubella HA antigens (8, 24). It appeared that alkaline buffer treatment may have inactivated the hemagglutinins of certain of the viruses. With the exception of measles antigens, little or no HA activity was demonstrable in either the extracts or the cells after extraction (but HA antigen was present in portions of cells disrupted by freezing and thawing or by sonication).

Hallauer and Kronauer (7) reported that hemagglutinins of reovirus types 1 and 2 could be extracted from intact, infected monolayers with alkaline buffers, but in the present study reovirus type 3 HA was not recovered by alkaline extraction of harvested, infected cells. These workers found that minimal amounts of adenovirus HA and infectious virus were released from intact monolayers by alkaline extraction, and this was noted with adenovirus HA antigens in our study. However, high levels of adenovirus CF antigen were extractable with alkaline buffers.

The importance was demonstrated of using seed virus of known titer and infecting cells at a multiplicity of at least one for production of viral serologic antigens. For CF antigens the use of a large infecting dose of virus is advantageous not only from the standpoint of producing more potent antigens but also from the standpoint of being able to harvest cultures soon after infection, since AC activity tends to increase with prolonged incubation of cell cultures.

It has previously been shown in this laboratory that higher titered HA antigens of coxsackievirus type A21 were produced in HeLa cell cultures infected at a high multiplicity of virus than in cultures infected with lower doses of virus (20). This was considered to be due, in part, to the more rapid destruction of host cells, preventing them from producing HA-inhibitory substances. On the other hand, it has been reported that the highest titered echovirus type 6 HA antigens were derived from MK cell cultures infected with low doses of virus (2). With our HA strain of echovirus type 6 the reverse was seen; the cells and fluids with the highest HA titers were those from cultures infected at a multiplicity of ten and harvested 2 days later.

In the case of reovirus type 3, the greatest amount of HA antigen was present in cultures infected at a multiplicity of ten, but at 5 days after infection it was demonstrable only in the cells. With lower infecting doses of virus, less total antigen was produced in the cultures, but more was released into the culture fluids. A delayed release of reovirus type 3 HA antigen into cell culture fluids has also been noted by Zalan and Labzoffsky (29), but the infecting virus doses used for their experiments were not indicated.

Although some general conditions for the production of high-titered viral serological antigens were defined by these studies, the investigations also emphasized that no single procedure is suitable for preparation of all viral antigens, and that it is necessary to systematically define the optimal conditions for preparation of each type of antigen.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Carol W. Shon and Barbara L. Thompson.

This study was supported by U.S. Public Health Service grant AI-01475 from the National Institute of Allergy and Infectious Diseases.

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