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Physicochemical and Serological Characteristics of Respiratory Virus Fluorescein-Isothiocyanate Conjugates for Fluorescent-Antibody Diagnosis

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Fluorescein-isothiocyanate (FITC) conjugates were prepared by improved methods from standard reference antisera to influenza A and B, mumps, parainfluenza 1, 2, 3, and 4, herpesvirus, respiratory syncytial virus, and adenovirus hexon. The antisera, prepared in a variety of animals, were fractionated three times with selected optimal concentrations of ammonium sulfate and yielded gamma globulins of adequate purity for conjugation with FITC. Conjugates containing optimal fluorescein-to-protein ratios of between 5 and 10 were produced in 2 h by dialysis labeling. Serological titers of each antiserum and conjugate were determined by complement fixation, hemagglutination-inhibition, serum neutralization, and indirect hemagglutination tests where appropriate. When corrected for dilution, the serological titers of the FITC conjugates were identical to those of the starting antisera. The fluorescent-antibody staining titers correlated well with one of the serological parameters of the original serum. The conjugates stained homologous antigens specifically and were free of nonspecific staining at the working dilution. Undesired staining of host cells which was a problem with some of the conjugates produced from sera containing cellular antibodies was removed by absorption with packed cells. These physicochemical and serological findings were then used as a guide in preparing high quality reagents for fluorescent-antibody identification of respiratory viruses.

Virus isolation and identification in the diagnostic laboratory is both an expensive and a tedious task. Many investigators, however, have shown that a sensitive and rapid viral diagnosis is often possible by means of immunofluorescent staining. Unfortunately, the application of this test to diagnostic virology has been limited by technically laborious test procedures and the inavailability of high quality reagents. Recently, the immunofluorescence test has been greatly facilitated by the use of improved microculture methods (17, 35, 36) and by the commercial availability of microculture slides and dishes. In addition, many methods have now been described for the improvement of immunofluorescence reagents and materials (1, 2, 4, 7-16, 18, 24-26, 28-33, 35-37).

In the following study, fluorescein-isothiocyanate (FITC) conjugates were prepared from standard reference antisera by improved methods of fractionation (13, 14) and conjugation (16). The physicochemical and serological characteristics of the conjugates were then evaluated. When these characteristics were known, the factors responsible for the conjugate deficiencies could often be identified and methods for improving the quality of the reagents could be applied. Microculture procedures were successfully used in these studies to simplify and accelerate the staining and microscopy observation of slides. Large numbers of slides were easily scanned under low and medium magnification with a dry darkfield condenser.

MATERIALS AND METHODS

Antisera. The antisera used for conjugation were obtained from the Respiratory Virology Unit or from the Scientific Research Branch, Center for Disease Control, Atlanta. All sera had initially been prepared and evaluated as standard reference sera for various serological tests. A brief description of the antisera is given in Table 1.

Fractionation of antisera. The major protein components of the sera were separated by ammonium sulfate precipitation carried out as described by Hebert et al. (13, 14). The gamma globulins from each serum were recovered by three precipitations with the optimum salt concentration (30 to 45%) as recommended (13, 14). Free ammonium sulfate was re-

TABLE 1. *Source of antisera used for fluorescent-antibody conjugation*

Antiserum	Animal host	Immunizing antigen		Inoculation route[a]
		Virus strain	Virus culture system	
Influenza A[b]	Guinea pig	A/Eq 2/Milford/2/63	Chick embryo (allantoic)	IN
		A/Swine/1976/31	Monkey kidney	IP, IM
Influenza B[b]	Guinea pig	B/Lee/40	Chick embryo (allantoic)	IN
		B/Mass/3/66	Monkey kidney	IP, IM
Mumps[c]	Guinea pig	Enders	Chick embryo (allantoic)	IN
Parainfluenza 1	Guinea pig	C-35	Chick embryo (amniotic)	IP
Parainfluenza 2	Rabbit	Greer	Primary human amnion	IV, IP
Parainfluenza 3	Guinea pig	C-243	African green monkey kidney	IP
Parainfluenza 4A	Guinea pig	M-25	Monkey kidney	IV, IP
Herpes simplex	Rabbit	VR-3 MacIntyre	Primary rabbit kidney	IP, IM
Respiratory syncytial	Mouse (ascitic fluid)	Long	HEp-2	IP
Adenovirus[d]	Rabbit	Hicks	HEp-2	IM

[a] Inoculation route: intranasal, IN; intramuscular, IM; intraperitoneal, IP; intravenous, IV.

[b] Immunizing antigens for influenza A and B antisera were absorbed with guinea pig or chicken erythrocytes to remove the hemagglutinating and serum neutralizing antigens and to leave only the soluble antigens suitable for preparing group-specific CF antisera.

[c] Antiserum to mumps viral and soluble antigens.

[d] Adenovirus group-specific antiserum to the AV9 hexon antigen, purified and prepared according to Hierholzer and Dowdle (19).

moved by overnight dialysis against saline. Protein concentrations of whole sera and of the gamma globulin-enriched fractions were determined by the biuret method (22) and measured at 560 nm in a Beckman DB-GT spectrophotometer. The protein composition or profile of each serum and globulin fraction was determined by cellulose acetate strip electrophoresis as described by Hebert et al. (14, 16).

Conjugation of serum globulins. FITC was obtained as a highly purified reagent from the Biological Reagents Section, Center for Disease Control. The sera were conjugated with FITC using a modification (16) of the dialysis method of Clark and Shepard (9). The final protein concentration of the conjugates was approximately 5 mg/ml. After conjugation, the free dye was removed either by dialysis against 0.01 M phosphate-buffered saline (PBS), pH 7.6, or by chromatography through a column (2.5 by 20 cm) of Sephadex G-25 medium equilibrated with the PBS buffer. Chromatographed conjugates were returned to the original concentration (5 mg/ml) by ultrafiltration with type PM-30 membranes (Amicon Corp., Lexington, Mass.).

Fluorescein and fluorescein-to-protein ratios. FITC concentration was determined as protein-bound FITC by absorbance at λ-max (near 495 nm) in 0.1 N NaOH, and the concentration was then related to a pure fluorescein diacetate reference standard (27). The fluorescein-to-protein (F/P) ratio was calculated as micrograms of FITC to milligrams of protein per milliliter of conjugate.

Virus propagation. Prototype virus strains were obtained from the Respiratory Virology Unit, Center for Disease Control, and were propagated in human epidermoid carcinoma (HEp-2) or primary rhesus monkey kidney cells in four-chambered microculture slides (Lab-Tek Products, Westmont, Ill.). Cultures were maintained in Eagle minimal essential medium with 100 U of penicillin, 50 μg of streptomycin, and 2 μg of amphotericin B per ml of culture fluid but without serum. All cell cultures were incubated at 35 C in an atmosphere of 5% CO_2 and 80% humidity.

Direct immunofluorescent staining. The maintenance medium was removed from the cell monolayers. The monolayers were rinsed three times in PBS and air dried. The cells were then fixed with acetone for 10 min at −20 C and stored at −70 C for staining at a later time. For staining, 0.05 ml of conjugate was applied to the cell monolayer, incubated for 45 min at 37 C in a moist chamber, and then rinsed for 10 min in each of two changes of PBS with a final quick rinse in distilled water. The slides were air dried and mounted in 90% glycerol/10% PBS.

Fluorescence microscopy. A Leitz fluorescence microscope equipped with an Osram HBO 200 mercury-vapor lamp and a 10×- and 25×-apochromat objective lens was used throughout the study. For excitation a UG 1 filter was used in conjunction with an ultraviolet-absorbing eyepiece barrier filter and a dry darkfield condenser. Photomicrographs were made with this system and a 35-mm Canon camera on high speed (ASA 400) Ektachrome daylight film at 60-s exposures.

Titration of virus globulin-FITC conjugates. The optimum working dilution of each globulin-FITC conjugate was determined by preparing dilutions in PBS and staining infected monolayer cultures with the various dilutions of the conjugate. The highest dilution of conjugate producing specific fluorescence with an intensity of 3 to 4+ and with a minimum of

background staining was selected as the optimum working dilution.

Specificity of conjugated globulins. The specificity of the conjugates was determined by the absence of specific staining of uninfected cell monolayers and of cells infected with heterologous viruses. Specific and heterologous antibody content of the conjugates to prototype virus strains were also measured by various serologic tests as appropriate for each antiserum. The sera and conjugates were heat inactivated at 56 C for 30 min for all serologic tests. Complement-fixing (CF) antibodies to influenza A and B, parainfluenza 1, 2, 3, 4A, and 4B, mumps "viral" antigen, herpesvirus types 1 and 2, respiratory syncytial virus (RSV), and the adenovirus (AV) group-specific hexon antigen (19) were assayed by the standardized CF test with overnight fixation of 5 U of complement (6). Hemagglutination-inhibiting (HI) antibodies to influenza A and B, parainfluenza 1, 2, 3, 4A, and 4B, mumps, and AV types 2, 8–10, 13, 15, 17, 19, 22–24, 26, and 27 were assayed. The standardized HI test was used with 0.01 M PBS diluent, pH 7.2, and with spectrophotometrically standardized 0.4% guinea pig erythrocytes for the myxoviruses and Sprague-Dawley rat erythrocytes for the adenoviruses (20, 21). Hemagglutinating antigens of parainfluenza 4A and 4B were prepared according to Killgore and Dowdle (23). Serum neutralizing antibodies to influenza A and B, parainfluenza 1, 2, 3, 4A, and 4B, mumps, herpes simplex virus type 1, RSV, and AV types 8–10, 13, 15, 17, 19, and 22 were determined with 30 to 70 tissue culture infectious doses of virus in monkey kidney (myxoviruses and adenoviruses), HEp-2 (RSV), or human embryonic lung cells (herpesvirus). Indirect hemagglutinating antibodies to herpesvirus types 1 and 2 were determined by the procedure of Bernstein and Stewart (3).

RESULTS

Antisera species. Influenza FITC conjugates were prepared from goat, guinea pig, horse, and rabbit antisera fractions. These conjugates yielded satisfactory specific staining titers without nonspecific staining (NSS). Mumps FITC conjugates prepared from antibody fractions of human convalescent sera, horse sera, or guinea pig sera were all equally satisfactory. Parainfluenza conjugates were prepared from antisera fractions obtained from goats, rabbits, guinea pigs, and horses. All of the parainfluenza conjugates except parainfluenza type 2 required absorption with normal monkey kidney cells for the removal of NSS. This NSS was removed by absorbing a 1:5 dilution of conjugate at a temperature of 37 C for 1 h with 0.1 ml of whole packed cells. Parainfluenza 4A (F/P 15.0) and RSV FITC conjugates required several absorptions. Parainfluenza 4A required multiple absorptions to fully remove the NSS. This conjugate, however, still had the highest staining titer of the parainfluenza group. A major problem with the parainfluenza sera was that many of the sera contained heterologous viral immunoglobulins, and attempts to remove these immunoglobulins lowered the specific staining titer.

Herpesvirus immunoglobulins were obtained from human convalescent antisera, guinea pigs, and rabbits. The only lot of antiserum satisfactory for conjugate production was obtained from rabbits immunized with herpesvirus cultured in rabbit cells. Since NSS was not present at the dilution used for staining no adsorption was required. RSV antibody was obtained from ferret and horse antisera and mouse ascitic fluids. Only the conjugates prepared from the mouse ascitic fluids yielded satisfactory staining, and these required multiple absorptions with whole HEp-2 cells to remove the NSS. Adenovirus FITC conjugates were prepared with antisera fractions obtained from humans, horses, and rabbits. The best results were obtained with labeled antisera from rabbits immunized with purified AV9 hexon antigen (19). Type-specific staining with AV9 virus was obtained at a 1:80 dilution of the conjugate, and group-specific staining was obtained at a dilution of 1:20. Nonspecific staining was not observed.

Physicochemical characteristics of sera and FITC conjugates. Each of the conjugates was fully characterized in physicochemical and immunofluorescence tests. For each virus, the conjugate which appeared to have the best staining characteristics was selected for further serologic studies. The physicochemical properties of these 10 superior FITC conjugates and their original antisera are shown in Table 2.

The conjugate protein concentrations varied from 4.5 to 5.5 mg/ml and the FITC concentrations varied from 24.4 to 74.9 μg/ml. The electrophoretic composition of the sera or serum fraction is also shown. Serum globulins precipitated a single time with ammonium sulfate contained large amounts (15 to 20%) of albumin. Conjugates prepared from these fractions often stained noninfected cells with the same intensity as virus-infected cells. Absorption of these conjugates with whole cells reduced specific staining as well as NSS. When these conjugates were refractionated twice more with optimal concentrations of ammonium sulfate, the albumin content was reduced to 2% or less and satisfactory staining was then obtained. Therefore, globulin fractions were obtained with the methods recommended by Hebert (13, 14). Hence, the gamma globulin content of the serum fractions shown in Table 2 varied

TABLE 2. *Physicochemical characteristics of sera and FITC conjugates*

Sera and FITC conjugates	Physicochemical characteristics					
	Protein (mg/ml)	FITC (μg/ml)	F/P ratio	Composition by case[a]		
				% Gamma	% Alpha-beta	% Albumin
Influenza A						
Serum (guinea pig)	51			8	48	44
Conjugate	5.0	34.0	6.8	25	75	0
Influenza B						
Serum (guinea pig)	53			6	51	43
Conjugate	4.5	34.0	7.5	36	64	0
Mumps						
Serum (guinea pig)	64			10	45	45
Conjugate	4.5	32.4	7.2	24	74	2
Parainfluenza 1						
Serum (guinea pig)	38			10	43	46
Conjugate	4.5	27.2	6.0	47	52	1
Parainfluenza 2						
Serum (rabbit)	68			28	23	49
Conjugate	5.0	44.0	8.8	70	30	0
Parainfluenza 3						
Serum (guinea pig)	44			6	43	50
Conjugate	5.0	45.5	9.1	52	48	0
Parainfluenza 4A						
Serum (guinea pig)	58			3	51	46
Conjugate	5.0	74.9	15.0	32	66	2
Herpesvirus						
Serum (rabbit)	70			12	26	62
Conjugate	5.0	24.4	4.9	77	23	0
RSV						
Ascitic fluid	36			12	29	59
Conjugate	5.5	54.8	10.0	58	42	0
Adenovirus						
Serum (rabbit)	68			16	33	51
Conjugate	5.0	29.6	5.9	61	37	2

[a] Case, Cellulose acetate strip electrophoresis. The electrophoretic profiles were determined on whole sera and on unconjugated serum fractions.

from 24 to 77% after three fractionations.

Predictable and reproducible F/P ratios were easily obtained with the dialysis method. When the serum protein concentration was adjusted to 5 mg/ml and dialyzed against a solution containing 0.125 mg of FITC/ml, F/P ratios of 2 to 5, 5 to 9, 10 to 12, and 24 to 35 were obtained in 1, 2, 4, and 24 h, respectively. The optimal F/P ratio for serum was determined by tagging pilot serum samples with differing F/P ratios and evaluating their staining characteristics. F/P ratios less than two failed to yield satisfactory staining, even at the lowest conjugate dilution (1:5). Conjugates with F/P ratios between 5 and 10 had the highest staining titers and least NSS. F/P ratios higher than 10 were satisfactory only with sera containing extremely high antibody titers. With these sera, NSS was eliminated by dilution. Increasing F/P ratios beyond 5 to 10 led to increasing NSS with no increase in specific staining.

Serological characteristics of sera and FITC conjugates. The serological characteristics of the sera and FITC conjugates (Table 2)

are shown in Table 3. Since the FITC conjugates were titrated on tissues containing known viruses and because the viral antigens usually had a characteristic distribution and appearance within the cell, a 1 to 2+ specific staining titer was determined in addition to the 3 to 4+ titer. When the 1 to 2+ specific fluorescent-antibody staining titer of a conjugate was compared with the other serological titers of that conjugate, it was found to directly correlate with the titer of the serologic test for which the serum was initially prepared (italicized titers). The actual fluorescent-antibody titer, however, was considerably influenced by the antigen slide preparation. Titrations on infected cell smears were often more sensitive than those on cell monolayers, but NSS was more frequent. Also, the predominating viral protein stained by the conjugate varied with the conjugate dilution.

The titers of the conjugates shown in Table 3 are actually identical to the initial serum titers if they are corrected for the dilution of gamma globulin. Therefore, the fluorescent-antibody titer of a conjugate could be predicted from either the CF, HI, or serum neutralizing titer of the antiserum.

The characteristic specific immunofluorescence obtained with selected conjugates is shown in Fig. 1 through 6. Figures 5a and b demonstrate the changes in immunofluorescence patterns with time. Figure 6a shows the value of low power for scanning monolayers, and Fig. 6b shows the value of high power for observing morphological detail.

The specificity of the FITC conjugates was confirmed by the absence of specific staining of uninfected cell monolayers and of cells infected with heterologous viruses (Table 4). Specific staining of cells infected with the heterologous viruses used in these tests did not occur at the conjugate dilutions used, except where the conjugates were intended as "group" reagents. For example, the influenza A conjugates stained all of the influenza A strains tested, and the B conjugates stained the B strains. Parainfluenza 4A conjugate stained parainfluenza 4A and 4B infected monolayers with equal intensity. Herpes simplex virus conjugate stained herpesvirus 1- and herpesvirus type 2-infected cells equally well. The AV9 antihexon conjugate at a dilution of 1:20 stained monolayers infected with each of the adenovirus types.

Specificity of antisera and those conjugates listed in Table 4 were also measured by CF, HI, and serum neutralizing tests in complete checkerboard fashion. Typical group and subgroup cross-reactions were present (e.g., among the myxoviruses); however, none of the heterologous CF, HI, and serum neutralizing titers observed with these sera and conjugates were outside the expected range.

DISCUSSION

The most important problems with the fluorescent antibody method in virology are the occurrence of NSS and undesired specific staining of host cells. These problems can be reduced by carefully choosing the immunizing antigens

TABLE 3. *Serological characteristics of sera and FITC conjugates*

Serum specificity	Homotypic titers									
	Antisera				FITC Conjugates					
	CF	HI	SN[a]	IHA[a]	CF	HI	SN[a]	IHA[a]	FA[a]	
Influenza A soluble	160	20[b]	<10[b]	—[c]	*40*	<10	<10	—	20[d]	*40*[e]
Influenza B soluble	80	20[b]	40[b]	—	*20*	<10	20	—	20	*40*
Mumps viral and soluble	320	160	1280	—	*80*	40	160	—	10	*40*
Parainfluenza 1	320	80	1280	—	40	*40*	320	—	10	*40*
Parainfluenza 2	320	320	640	—	40	*40*	160	—	20	*40*
Parainfluenza 3	80	160	160	—	40	*80*	80	—	10	*40*
Parainfluenza 4A	640	160	1280	—	20	*40*	640	—	40	*80*
Herpes simplex type 1	640	—	160	160	160	—	*40*	40	10	*40*
RSV (ascitic fluid)	160	—	160	—	*80*	—	80	—	10	*20*
Adenovirus antihexon	320[f]	1280[g]	40[g]	—	*80*	160	20	—	80	*160*

[a] SN, Serum neutralizing; IHA, indirect hemagglutinating; FA, fluorescent-antibody.

[b] Homotypic HI and SN antibodies in the influenza A and B sera and conjugates are intentionally low or undetectable as a result of the immunizing antigen preparation (see Table 1, footnote *a*).

[c] —, Not tested.

[d] Titers obtained using 3 to 4 + intensity of fluorescence.

[e] Titers obtained using 1 to 2 + intensity of fluorescence.

[f] Group-specific CF antibody measured with purified AV2 hexon antigen.

[g] Type-specific HI & SN antibodies measured with crude AV9 virus.

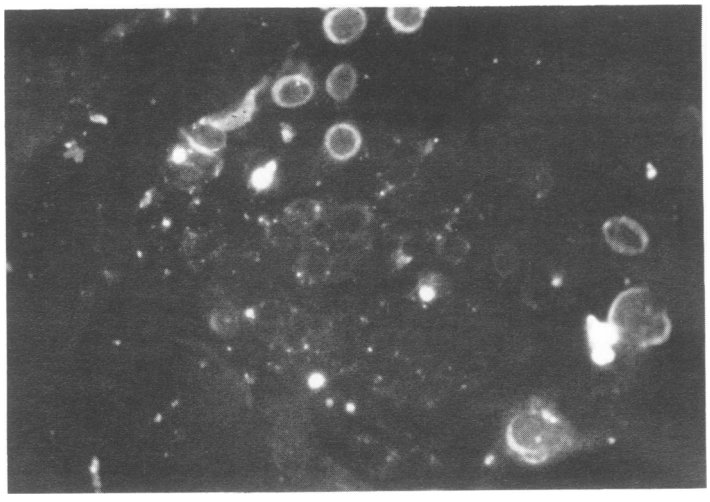

FIG. 1. *Rhesus monkey kidney cell monolayer infected with parainfluenza 1 and stained with parainfluenza 1 FITC conjugate* (×216).

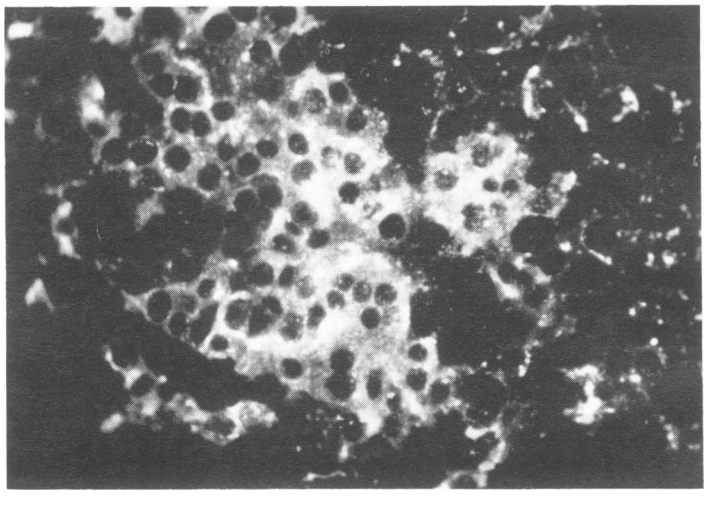

FIG. 2. *Rhesus monkey kidney cell monolayer infected with parainfluenza 2 and stained with parainfluenza 2 FITC conjugate* (×216).

used for antiserum production and by conjugating the gamma globulin fractions under satisfactorily controlled conditions. Pure viral protein immunogens are now available for selected viruses. When these preparations are not available, however, crude or semipurified viral immunogens should be prepared whenever possible from tissues homologous to the animal immunized. The important features of FITC conjugation are to obtain gamma globulins as free of other serum proteins as possible and to label the gamma globulins with the optimum number of fluorescein molecules. Although the use of pure gamma globulins or even specific antibodies themselves may be the ultimate goal, compromises are presently necessary for the sake of practicality.

In our laboratory, the use of the optimal ammonium sulfate concentrations recently recommended by Hebert (13, 14) proved to be simple and to yield satisfactory serum proteins for conjugation with FITC. Hebert's procedures differ from previous ones in that the concentration of ammonium sulfate is varied according to the serum species fractionated. This method can easily be done in most laboratories, and the ammonium sulfate can readily be removed by overnight dialysis. The protein fractions are then ready for labeling with FITC the following day. Under controlled conditions (16, 26) serum

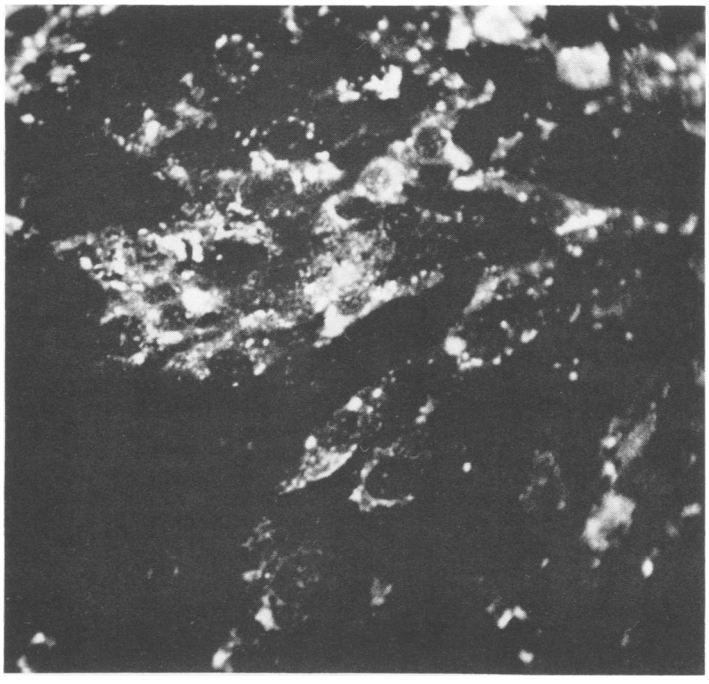

FIG. 3. *Rhesus monkey kidney cell monolayer infected with parainfluenza 4A and stained with parainfluenza 4A FITC conjugate* (×216).

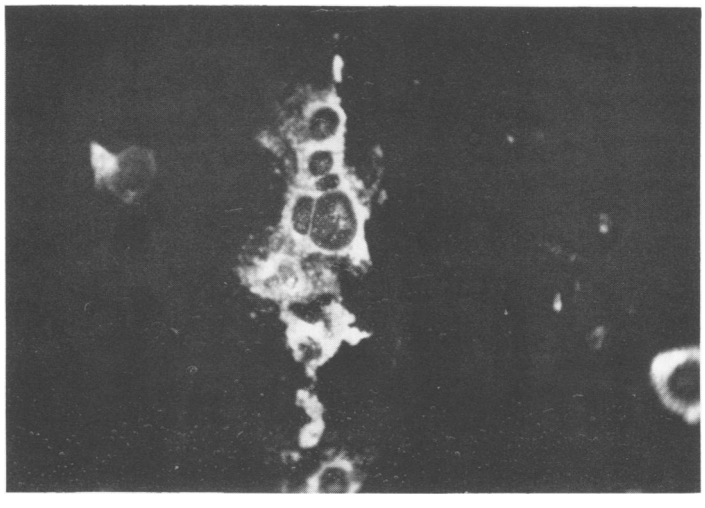

FIG. 4. *Rhesus monkey kidney cell monolayer infected with mumps and stained with mumps FITC conjugate* (×216).

proteins were labeled with selected F/P ratios by the dialysis method (9, 16). The labeling rate and efficiency were found to be influenced both by the source of the antiserum and by the relative concentrations of serum proteins (albumin, beta globulins, alpha globulins, gamma globulins, etc.). Gamma globulins containing an optimum F/P ratio (5 to 10) were produced in 2 h. Increasing the F/P ratios beyond this optimum range did not increase the specific staining titer of the conjugate but did increase the NSS. Predetermination of the optimum value for each antiserum fraction is essential for high quality conjugate production.

Most of the antisera obtained for these studies were prepared with viral antigens produced

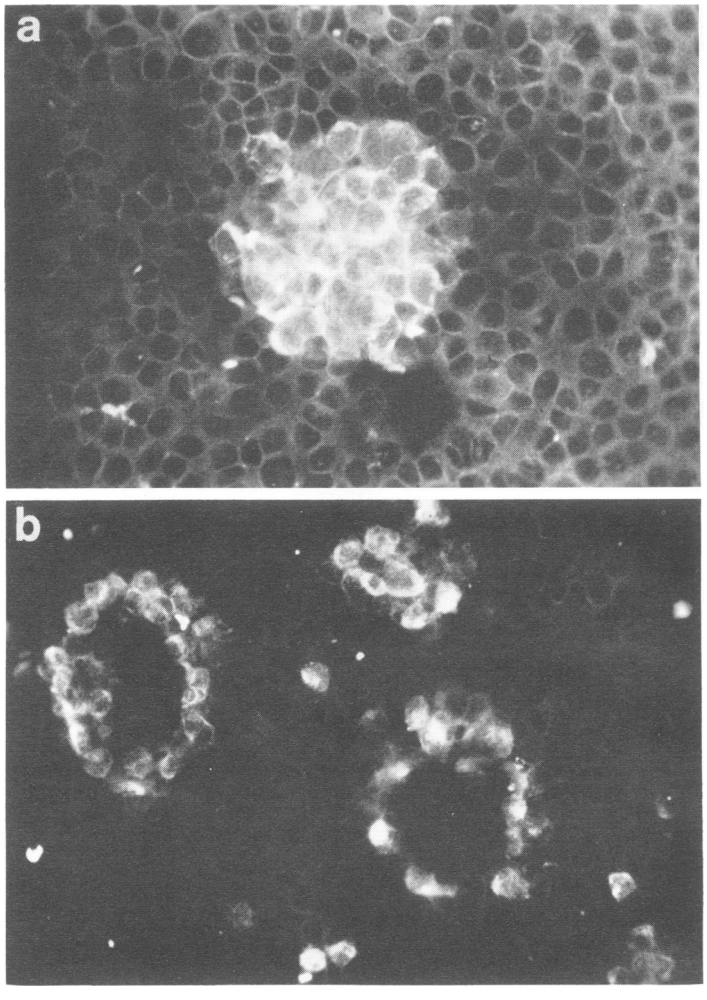

FIG. 5. *HEp-2 cells infected with herpesvirus (×216). (a) Monolayer stained with herpesvirus FITC conjugate 24 h after infection. (b) Monolayer stained with herpesvirus FITC conjugate 72 h after infection.*

in heterologous tissues. Undesired specific tissue staining with these conjugates was removed by dilution or absorption of the conjugate with intact cells. However, absorption almost always lowered the specific staining titer of the conjugate.

These studies were facilitated by using multichambered microculture slides. Cells were grown, infected, fixed, and stained directly on these glass microculture slides (1 by 3 inch [ca. 2.54 by 7.62 cm]). Leaving dividing gaskets in place throughout the staining procedure made it possible to stain the preparations with several different FITC conjugates at one time. Large numbers (50 per day) of these slides (four preparations per slide) were easily scanned under low (10×) and medium (25×) magnification with a dry darkfield condenser.

For the evaluation of conjugate staining characteristics the preparation of positive and negative control slides was critical. Carter (5) has reviewed in detail the problem of demonstrating the viral antigen in cell monolayers. Monolayers with extensive cytopathic effect should be avoided since NSS is frequently increased. Suitable control slides were prepared in our study after preliminary experiments in which monolayers were infected with a range of virus concentrations and incubated for various time intervals.

The serological titers of the sera were the same as those of the conjugates when the resulting titers were corrected for dilution of the gamma globulins. In diagnostic tests, the FITC conjugates were sometimes used at lower dilutions to better identify a morphological charac-

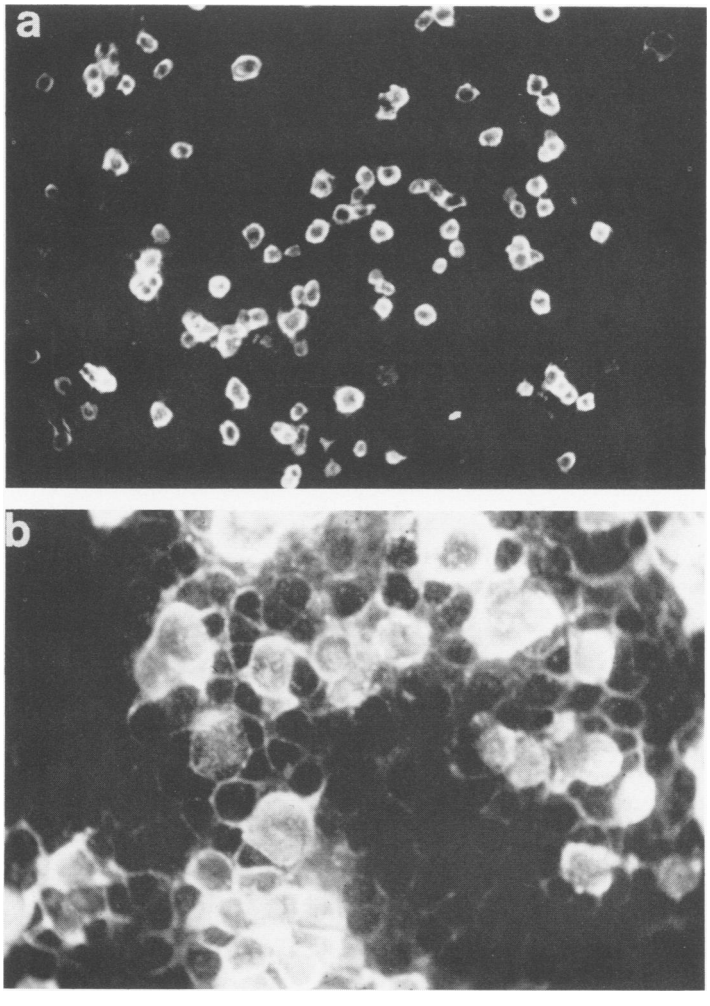

FIG. 6. *HEp-2 cells infected with AV 9 and stained with adenovirus antihexon FITC conjugate. (a) 10× low power objective (×86). (b) High power objective (×216).*

TABLE 4. *Staining specificity of FITC conjugates*

Virus antigen	FITC conjugates and dilution used									
	Influenza A (1:20)	Influenza B (1:20)	Mumps (1:10)	Parainfluenza 1 (1:10)	Parainfluenza 2 (1:20)	Parainfluenza 3 (1:10)	Parainfluenza 4A (1:40)	Herpesvirus (1:10)	RSV (1:10)	Adenovirus (1:20)
Influenza A[a]	3	0	0	0	0	0	0	0	0	0
Influenza B[a]	0	3	0	0	0	0	0	0	0	0
Mumps	0	0	4	0	0	0	0	0	0	0
Parainfluenza 1	0	0	0	3	0	0	0	0	0	0
Parainfluenza 2	0	0	0	0	4	0	0	0	0	0
Parainfluenza 3	0	0	0	0	0	3	0	0	0	0
Parainfluenza 4A	0	0	0	0	0	0	4	0	0	0
Parainfluenza 4B	0	0	0	0	0	0	4	0	0	0
Herpesvirus 1	0	0	0	0	0	0	0	4	0	0
Herpesvirus 2	0	0	0	0	0	0	0	4	0	0
RSV	0	0	0	0	0	0	0	0	4	0
AV types 2, 3, 5, 7, 9	0	0	0	0	0	0	0	0	0	*4*
Noninfected HEp-2 cells	0	0	0	0	0	0	0	0	0	0
Noninfected Rhesus monkey kidney cells	0	0	0	0	0	0	0	0	0	0

[a] Several strains tested.

teristic and, as in the adenovirus FITC conjugate, to identify heterologous virus types. For example, the AV9 antihexon conjugate stained AV9 at a dilution of 1:80, but group-specific antigens (AV2 and AV7) were detected best with a 1:20 dilution. This conjugate was useful for detecting adenovirus infections of each antigenic type.

The fact that fluorescent-antibody staining titers do not always agree with serological titers has been noted in the literature (10, 18). In our laboratory we have observed a close relationship between fluorescent-antibody titers and the serological titer determined by the test for which the sera was initially prepared. The actual conjugate titers, however, are influenced by the antigen slide preparations and the predominating antigens present. Still, when the serological titers and the physiocochemical characteristics of sera were known, it was generally possible to predict the serological and immunofluorescence titers of the conjugates.

Knowledge of the physicochemical and serological characteristics of immunofluorescence reagents has supplied important information in our studies for correlating reagent performance. Limited field trials, currently in progress with the reagents produced by these methods, have proved them to be successful in detecting viral antigens in infected tissues rapidly and specifically.

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