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Comparison of Chemicon SimulFluor Direct Fluorescent Antibody Staining with Cell Culture and Shell Vial Direct Immunoperoxidase Staining for Detection of Herpes Simplex Virus and with Cytospin Direct Immunofluorescence Staining for Detection of Varicella-Zoster Virus

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A new rapid direct immunofluorescence assay, the SimulFluor direct fluorescent-antibody (DFA) assay, which can simultaneously detect herpes simplex virus types 1 and 2 (HSV-1 and -2) and varicella-zoster virus (VZV), was evaluated in comparison with our current standard procedures of (i) shell vial direct immunoperoxidase (shell vial IP) staining and cell culture for detection of HSV and (ii) cytospin DFA staining for VZV detection. A total of 517 vesicular, oral, genital, and skin lesion specimens were tested by all three procedures. For HSV detection, the SimulFluor DFA assay had an overall sensitivity, specificity, positive predictive value, and negative predictive value of 80.0, 98.3, 92.3, and 95.1%, respectively, when compared to culture. Shell vial IP staining had a sensitivity, specificity, positive predictive value, and negative predictive value of 87.6, 100, 100, and 96.9%, respectively, when compared with cell culture. The SimulFluor DFA assay, however, offers same-day, 1.5-hours results versus a 1- to 2-day wait for shell vial IP staining results and a 1- to 6-day wait for culture results for HSV. For VZV detection SimulFluor DFA staining detected 27 positive specimens as compared to 31 by our standard cytospin DFA technique—a correlation of 87.1%. A positive SimulFluor reaction for VZV is indicated by yellow-gold fluorescence compared to the bright apple-green fluorescence observed by cytospin DFA staining. There is no difference in turnaround time between the two assays. The SimulFluor DFA assay is a rapid immunofluorescence assay that can detect 80% of the HSV-positive specimens and 87% of the VZV-positive specimens with a 1.5-h turnaround time.

Herpes simplex virus (HSV) and varicella-zoster virus (VZV) cause skin lesions in adults and children and may cause severe systemic disease in immunosuppressed hosts and neonates. HSV types 1 and 2 (HSV-1 and -2) can cause vesicular and ulcerative lesions on the genital area as well as oropharyngeal infection. Genital herpes infection is a public health concern, as the infection can be transmitted between sexual partners. Seroprevalence studies of herpes type-specific antibodies have shown an increase of over 30% in the prevalence of HSV-2 infections over the past two decades, with a nationwide incidence of more than 20% of those infected who are 12 years of age and older having detectable antibody to HSV-2 (2). HSV-1 is increasingly recognized as a cause of genital infection, especially in female patients. In the United Kingdom, the annual incidence of HSV-1 genital infection nearly tripled over a 7-period with an incidence of 79% found in one study (8). Most patients with genital herpes infection do not have symptoms and thus are not aware that they can infect their sex partners. Another concern with genital herpes is neonatal herpes. Pregnant women who acquire primary genital herpes shortly before labor are the ones most likely to infect the newborn (1).

Herpes zoster virus is a common childhood disease and is also a serious infection in the elderly and immunocompromised patients. In the United States, before the VZV vaccine was available about 100 healthy people died from chicken pox annually, half of whom were children and the other half of whom were adults. Also, approximately 11,000 people were hospitalized annually for complications from varicella (3). Chicken pox can be a fatal disease during pregnancy as well as in human immunodeficiency virus-positive patients.

Currently, most clinical virology laboratories use cell culture for detection of these two herpesviruses. Cell culture usually requires several days before results can be reported. Some laboratories use a PCR assay to detect herpesviruses in cerebrospinal fluid and in other tissues. PCR has the advantages of a higher sensitivity and a shorter turnaround time than those of culture. But none of these PCR assays can currently be used in the clinical laboratory in large scale, nor do they have the same-day turnaround time of a couple of hours of the SimulFluor DFA assay. In situations such as labor and delivery, a rapid assay may be required if a woman is suspected of having a primary genital infection. In this study, we evaluated SimulFluor direct fluorescent-antibody (DFA) staining for detection of HSV in comparison with our current shell vial immunoperoxidase (IP) staining and with cell culture. We also compared SimulFluor DFA staining with our current cytospin DFA staining method for VZV detection for sensitivity, specificity, and predictive values and for turnaround time for results.

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TABLE 1. Distribution of viruses detected by each assay

Subjects (no.), and specimen type (no.)	Virus(es) detected (no. of samples) by:			
	SimulFluor DFA staining	Shell vial IP staining	Culture	VZV cytospin-DFA
Male (178)				
Genital (113)	HSV-1 (7), HSV-2 (12), VZV (2)	HSV-NT[a] (20)	HSV-1 (7), HSV-2 (14)	VZV (2)
Oral (33)	HSV-1 (6)	HSV-NT (6)	HSV-1 (9)	VZV (1)
Other (32)	HSV-1 (2), VZV (7)	HSV-NT (2)	HSV-1 (2)	VZV (8)
Female (339)				
Genital (225)	HSV-1 (29), HSV-2 (13), VZV (4)	HSV-NT(43)	HSV-1 (34), HSV-2 (18)	VZV (4)
Oral (34)	HSV-1 (11)	HSV-NT (15)	HSV-1 (15)	
Other (80)	HSV-1 (3), HSV-2 (1), VZV (14)	HSV-NT (6)	HSV-1 (4), HSV-2 (2)	VZV (16)

[a] NT, nontypeable.

MATERIALS AND METHODS

Samples. A total of 517 consecutive specimens, including 338 genital swabs, 67 oral swabs, and 112 swabs from other body sites, were submitted to the clinical virology laboratory at the Provincial Laboratory, Regina, Saskatchewan, Canada, over a 5-month period for routine testing for HSV and VZV. Cell culture, shell viral IP staining, and SimulFluor DFA staining were performed for detection of HSV on specimens as requested, while samples were tested for VZV with the cytospin DFA staining and SimulFluor DFA staining methods.

Slide preparation for SimulFluor DFA staining. Swabs in viral transport media were vortexed, wrung out, and discarded. One milliliter of the sample was then centrifuged at 10,000 rpm in an Eppendorf 5403 centrifuge for 10 min to pellet the cells. The cell pellets were resuspended in a small amount of phosphate-buffered saline, (PBS) and 25 μl of the suspension was added to one well of a Shandon multispot microscope slide. The slide was air dried and fixed in cold acetone for 10 min.

SimulFluor DFA staining. Cell spots on each slide were stained with 25 μl of SimulFluor DFA reagent (Chemicon International, Temecula, Calif.) for 30 min at 37°C in a humid chamber. The slide was washed gently for 15 s in PBS, mounted in Tris-buffered glycerin, and examined at ×100 magnification with fluorescence microscope (Olympus, Mississauga, Ontario, Canada). When a fluorescein isothiocyanate filter set was used, the primary component, containing monoclonal antibodies specific for HSV-1 and -2, bound to a 155-kDa major capsid protein in HSV-infected cells, resulting in an apple-green fluorescence, and the VZV antigen-antibody complex will fluoresce yellow-gold. When tetramethyl rhodamine isothiocyanate filter was used, the secondary component, containing rhodamine-labeled monoclonal antibodies specific for VZV, bound to the glycoprotein gp1 and the immediate early antigen in VZV-infected cells, resulting in a hot pink fluorescence.

Cytospin DFA staining for VZV testing. A 200-μl portion of the vortexed sample was added to the cup of the cytospin instrument for cytocentrifugation (Cytospin 2; Shandon Inc., Pittsburgh, Pa.) at 1,500 rpm for 5 min. The slide was air dried and fixed in cold acetone for 10 min. The cell spots were stained with 40 μl of Merifluor VZV immunofluorescence reagent (Meridian Diagnostics) for 30 min at 37°C in a humid chamber. Following a 15-s wash in PBS, the slides were mounted in glycerol and examined with a fluorescence microscope at a wavelength of 490 nm; the VZV antigen-antibody complex exhibited an apple-green fluorescence.

Shell vial IP staining. Two vials of Vero cell monolayers were stained, one at 24 to and one at 48 h postinoculation. The Vero cell monolayers were rinsed twice with Hanks balanced salt solution, fixed in cold acetone for 10 min, and allowed to air dry. The monolayers were covered with 200 μl of a 1:100 dilution of working conjugate, namely, HSV-2 horseradish peroxidase-conjugated antiserum (DAKO Corp., Santa Barbara, Calif); incubated for 60 min at room temperature; rinsed twice with distilled water; reacted with 200 μl of the appropriately diluted substrate 3-amino-9-ethylcarbazole (Sigma Chemical Co., St. Louis, Mo.); and incubated at 37°C for 30 min. After staining, each coverslip was rinsed with distilled water, mounted in 1 drop of Glycergel (DAKO Corp.), and examined under a light microscope for infected foci. Shell vial IP staining will react to both HSV-1 and HSV-2. Positive HSV-1 and -2 controls, as well as negative controls, were processed with each group of specimens in a similar manner.

Typing for HSV. A positive result for the HSV-VZV DFA staining procedure was indicated by the presence of two or more intact cells exhibiting specific fluorescence. When a fluorescein isothiocyanate filter set is used, the HSV antigen-antibody complex exhibits an apple-green fluorescence, and the VZV antigen-antibody complex exhibits yellow-gold fluorescence. For those specimens positive for HSV, an identification slide was prepared by adding 25 μl of the original cell suspension to each of two wells of a Shandon multispot microscope slide. The slide was air dried and then fixed in cold acetone for 10 min. Using the PathoDx herpes typing kit (InterMedico), one cell spot was stained with 25 μl of HSV-1 typing reagent and the other cell spot was stained with HSV-2 typing reagent for 30 min at 37°C in a humid chamber. The slide was washed gently for 15 s in PBS, mounted in buffered glycerol, and examined with a fluorescence microscope.

Virus isolation. For viral culture, an aliquot of each sample was obtained prior to centrifugation to pellet cells, inoculated into a Vero cell monolayer, incubated at 37°C, and examined for cytopathic effects daily for 6 days. Isolates were identified by immunofluorescence, using the PathoDx reagents described above.

RESULTS

Using virus isolation (culture) as the "gold standard," a specimen was classified as false positive for a particular assay if its result was positive and the results of the other assay and culture were both negative. Similarly, a specimen was classified as false negative if its result for a particular assay was negative and the results of the other assay and culture were both positive.

Of the 517 specimens tested for HSV, there were 35 discrepant specimens. Ten specimens were classified as false positive and 19 specimens were classified as false negative by SimulFluor DFA staining. Eleven specimens were classified as false negative by shell viral IP staining. Table 1 shows both the distribution of the virus in each assay and the virus detected. In the case of genital herpes, 33% of the infections in males were caused by HSV-1, while 65% of the female genital infections were caused by HSV-1. Table 2 shows the sensitivity, specificity, positive predictive value, and negative predictive value for both sexes, individually and in combination. The sensitivity of shell vial IP staining was slightly better than that of SimulFluor DFA staining, but the specificities for the two assays were almost identical.

Thirty-one specimens were positive for VZV by our current method of cytospin DFA staining, while 27 specimens were positive for VZV by SimulFluor DFA staining a correlation of 87.1%. Of the 27 positive results by the SimulFluor DFA assay, some were very difficult to read, as the cells exhibited more of an amber-red fluorescence than the yellow-gold fluorescence that they were supposed to exhibit. Furthermore, when these specimens were cytospun according to the protocol for detecting VZV described above, fewer cells stained positive by the

TABLE 2. Sensitivity, specificity, and positive and negative predictive values of SimulFluor DFA and shell vial IP assays in comparison with cell culture for both sexes, individually and in combination

Subject group (no. of specimens)	Assay	No. of specimens				Sensitivity (%)	Specificity (%)	PPV[a] (%)	NPV[a] (%)
		True positive	True negative	False negative	False positive				
Males (178)	SimulFluor DFA	27	141	5	5	84.4	96.6	84.4	96.6
	Shell vial IP	29	146	3	0	90.6	100	100	97.9
	Culture	32	146	0	0	100	100	100	100
Females (339)	SimulFluor DFA	57	264	16	2	78.1	92.2	96.6	94.3
	Shell vial IP	63	266	10	0	86.3	100	100	96.4
	Culture	73	266	0	0	100	100	100	100
Males + females (517)	SimulFluor DFA	84	405	21	7	80.0	98.3	92.3	95.1
	Shell vial IP	92	412	13	0	87.6	100	100	96.9
	Culture	105	412	0	0	100	100	100	100

[a] PPV, positive predictive value; NPV, negative predictive value.

SimulFluor DFA assay than with the Meridian Merifluor VZV immunofluorescence reagent.

Table 3 shows the turnaround time for each assay. SimulFluor DFA staining was able to detect 80% of the specimens positive for HSV on the same day, within 1.5 h of receipt of the samples. Shell vial IP staining detected only 41% of the positives within 24 h but 87% within 48 h. In order to detect close to 80% of the positive specimens, the cell culture assay would require 72 h.

DISCUSSION

Genital HSV-1 and -2 infections are on the rise in the United States as well as in other countries. Seroprevalence studies in the United States as well as in the United Kingdom have shown major increases in prevalences compared to those of 10 to 20 years ago (2, 8). This makes genital HSV infection the leading cause of viral sexually transmitted disease. In the U.S. study, seropositivity quintupled among white teenagers and doubled among whites in their 20s (2). Similar studies in the United Kingdom also demonstrated that genital HSV-1 infection is on a rapid rise, especially among women (7). Most of the patients in these studies did not realize that they had genital herpes infection. In addition, patients with genital herpes infection shed the virus in their genital tract even when symptoms were absent. In one study, transmission of more than 80% of the genital herpes infections occurred during subclinical reactivation (6). This posed two important public health problems: first, infected individuals could infect their sex partners, second, female patients could transmit the virus to their infants.

One suggestion for prevention of neonatal herpes infection is to screen the mother for HSV-2 antibody (4). However, currently there is no good commercially available kit that can differentiate HSV-1 from HSV-2. Also, the presence of HSV-1 antibody does not eliminate genital herpes, as more females have genital infection with HSV-1. In Saskatchewan, close to 50% of the genital herpes isolates from females are HSV-1, as indicated in another study (E. Chan and K. Brandt, unpublished data). Cell culture would be too time-consuming for patients already in the labor and delivery stages of childbirth. PCR assay would probably also not be useful in this situation, because 4 to 6 h are required to obtain results by that method. Also, the PCR test is so labor-intensive that most laboratories would not be able to perform it on an immediate basis.

A simple and rapid for test use in a clinical laboratory would be ideal for this situation, especially if the clinical disease is one that can be modified with antiviral treatment when detected early. The present study is designed to determine both whether SimulFluor DFA staining can be used as a rapid test and how its results compare with those of cell culture and the shell vial IP assay. Since the SimulFluor DFA assay can detect VZV and HSV simultaneously, we also evaluated whether our currently used cytospin DFA assay was the same capability.

The SimulFluor DFA assay was able to detect HSV directly in 84 of the 105 clinical specimens with a sensitivity of 80% (Table 2), which is slightly lower than that of the 2-day shell vial IP assay (87.6%). The SimulFluor DFA assay has a specificity of 98.3% and positive and negative predictive values of 92.3 and 95.1%, respectively, in comparison with the 100% values of cell culture (Table 2). The false-positive specimens observed with the SimulFluor DFA assay are probably a re-

TABLE 3. Turnaround time for detection of HSV from specimens

Assay	No. of specimens positive on indicated day of report[a]						
	S[b]	1	2	3	4	5	6
SimulFluor DFA	84 (80)						
Shell vial IP		43 (41)	49 (87.6)				
Culture		17 (16.2)	34 (48.6)	29 (76.2)	16 (91.4)	6 (97.1)	3 (100)

[a] Values in parentheses indicate cumulative percentages of positive specimens.
[b] S, same-day result.

flection of inexperience on the part of the technologists reading the slides. These false positives occurred early in the study and were not evident later on as the technologists gained experience. The specificity and the positive predictive value of the assay transfer should improve over time. In our study, the HSV detection results of the SimulFluor DFA assay did not show the same sensitivity as that achieved by Landry et al. (5), whose result surpassed that of cell culture. This could be explained by differences between the two studies in slide preparation. The study of Landry et al. used cytospin for slide preparation, while the present study used cell pellet spots in order to provide two spots on each slide for detection of each of the two viruses; differing sensitivities may have resulted. A study using cytospin for slide preparation is currently under way in our laboratory.

The overall performance of the SimulFluor DFA assay is very similar to that of the 48-h shell vial IP assay, with a difference in turnaround time between the two methods. SimulFluor DFA staining can be finished within 1.5 h of receipt of a specimen, while shell viral IP staining will takes at least 24 h to detect 41.7% of the positives and 48 h to detect 87.6% of the positives. Cell culture takes 2 days to detect 48.6% of the positives and 3 days to detect 76.2% of the positives (Table 3). The SimulFluor DFA assay is the only assay that can be used in cases where rapid detection of HSV or VZV is required. The present study is biased toward patients with skin lesions; how the SimulFluor DFA assay will perform with patients without lesions requires further study.

The SimulFluor DFA assay did not work as well for VZV detection as it did for HSV detection—the staining was not as evident and the number of positive cells was not as large compared to our standard method. SimulFluor DFA staining detected only 87.1% of the infected samples that our current method detected. Although the number of VZV-positive specimens in the study was small ($n = 31$), six of the positives were from genital specimens, leading us to ask what the role is of VZV in causing genital infection?. A further study to answer this question is under way in this laboratory. The SimulFluor DFA assay would definitely be useful in determining the prevalence of genital VZV infection in our patient population.

In conclusion, we found SimulFluor DFA staining to be a simple assay for the detection of HSV and VZV in clinical specimens, with a 1.5-h turnaround time after the initial training of technologist.

REFERENCES

1. **Brown, Z. A., S. Selke, J. Zeh, J. Kopelman, A. Maslow, R. L. Ashley, D. H. Watts, S. Berry, M. Herd, and L. Corey.** 1997. The acquisition of herpes simplex virus during pregnancy. N. Engl. J. Med. **337:**509–515.
2. **Fleming, D. T., G. M. McQuillan, R. E. Johnson, A. J. Nahmias, S. O. Aral, F. K. Lee, and M. E. St. Louis.** 1997. Herpes simplex virus type 2 in the United States, 1976 to 1994. N. Engl. J. Med. **337:**1105–1111.
3. **Gershon, A. A.** 2000. The varicella vaccine: from promise to practice. Infect. Dis. Clin. Pract. **9:**253–256.
4. **Gibbs, R. S., and P. B. Mead.** 1992. Preventing neonatal herpes—current strategies. N. Engl. J. Med. **326:**946–947.
5. **Landry, M. L., D. Ferguson, and J. Wlochowski.** 1997. Detection of herpes simplex virus in clinical specimens by cytospin-enhanced direct immunofluorescence. J. Clin. Microbiol. **35:**302–304.
6. **Mertz, G. J., J. Benedetti, R. Ashely, S. Selke, and L. Corey.** 1992. Risk factors for sexual transmission of genital herpes. Ann. Intern. Med. **116:**197–202.
7. **Ross, J. D. C., I. W. Smith, and R. A. Elton.** 1993. The epidemiology of herpes simplex type 1 infection of the genital tract in Edinburgh. Genitourin. Med. **69:**381–383.
8. **Tayal, S. C., and R. S. Pattman.** 1994. High prevalence of herpes simplex virus type 1 in female anogenital herpes simplex in Newcastle upon Tyne 1983-92. Int. J. STD AIDS **5:**359–361.