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Utility of Direct Immunofluorescence and Virus Culture for Detection of Varicella-Zoster Virus in Skin Lesions

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A direct immunofluorescence assay (DFA) with a monoclonal antibody from Ortho Diagnostic Systems was compared with conventional cell culture for the rapid detection of varicella-zoster virus (VZV) in 140 dermal lesions from 133 patients. A total of 79 (56%) specimens were positive for VZV: 40 (51%) by DFA alone, 2 (3%) by culture only, and 37 (47%) by both culture and DFA. After discordant analysis, the sensitivities and negative predictive values, respectively, were 97.5% (77 of 79) and 96.8% (61 of 63) for DFA and 49.4% (39 of 79) and 60.4% (61 of 101) for viral culture. Of the 39 positive viral cultures, VZV was isolated from 38 (97%) cultures in A549 cells, 23 (59%) in primary rhesus monkey kidney cells, and only 16 (41%) in MRC-5 cells. We conclude that DFA is the optimal method for rapid identification of VZV. In addition, better recovery of VZV in culture may be achieved by using A549 cells.

Infection with varicella-zoster virus (VZV) is a common cause of hospitalization among immunocompromised patients (3, 22). Individuals with deficiencies in cell-mediated immunity, such as those with AIDS or hematologic malignancies, are especially vulnerable to severe and fatal disease (5, 7, 10, 12, 19). Because the distribution and character of lesions in these patients are often atypical, laboratory studies can play a significant role in the accurate diagnosis of VZV infection. Rapid and sensitive detection of VZV from clinical specimens is important to assist in the prompt identification of patients most likely to benefit from antiviral therapy (2, 4) and to institute appropriate isolation procedures.

Viral isolation in conventional cell culture is considered to be the definitive diagnostic test for the detection of VZV. However, growing VZV in culture can be difficult and is usually too slow to be clinically useful (6, 16, 18). The efficiency of viral culture can be improved by employing a centrifugation-assisted shell vial culture system (9, 17, 23), but this method can be technically demanding and can still take several days to obtain a positive result. Nonculture methods, such as the Tzanck test (8, 16, 20), electron microscopy (8, 21, 24), and PCR (11), have been reported to be more rapid and more sensitive than culture for detecting VZV, but these methods have not been widely employed. Recent infection can also be confirmed by demonstrating significant rises in VZV-specific antibody titers (1). Because of inherent delays, serologic tests have had limited diagnostic utility.

Rapid detection of VZV by using specific fluorescent-antibody conjugates in a direct immunofluorescence assay (DFA) is also possible. Studies performed with VZV-specific polyclonal (6, 13, 18) or monoclonal (9, 14–16) antibodies have shown DFA to be a practical and useful method for the direct detection of VZV antigens from cell scrapings. With the present study, we summarize 64 months of our clinical experience in utilizing both viral isolation techniques and DFA for the rapid detection of VZV from skin lesions. In addition, viral culture results were reviewed in an attempt to identify factors which may enhance the recovery of the virus in tissue culture.

From 1 January 1988 through 30 April 1993, a total of 140 specimens from 133 patients with clinical signs suggestive of primary or reactivated VZV infection were simultaneously evaluated by virus culture and DFA. One hundred eight specimens were obtained from 103 pediatric patients seen at Children's Hospital of Philadelphia, while 32 specimens were from 30 employees of the Children's Hospital of Philadelphia and adult patients of nearby hospitals. All specimens for viral culture and slides for DFA were obtained and prepared by each patient's physician from identified skin lesions. Verbal and written instructions for the appropriate methods to collect and transport specimens were distributed to physicians as part of a continuing education program provided by the Clinical Virology Laboratory at Children's Hospital of Philadelphia. Physicians were instructed to unroof a fresh vesicular lesion and vigorously swab the base of the vesicle with a rayon or Dacron plastic-shafted swab to obtain cells. If no unruptured vesicles were present, a swab was obtained from the base of an ulcer. Cellular material from one swab was immediately rolled onto an ethanol-cleaned glass slide in two areas, each approximately 1 cm in diameter, and allowed to air dry. A companion swab was obtained for viral culture from either the same or a nearby lesion and immediately placed in 2.0 ml of viral-chlamydial transport medium (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.) and stored at 4°C until processed.

Slides for DFA were immediately fixed in cold (−20°C) acetone for 10 min and air dried, and 25 μl of a fluorescein isothiocyanate-conjugated mouse monoclonal antibody (3B3) specific for the VZV glycoprotein complex gp98-gp62 (Ortho Diagnostic Systems, Inc., Raritan, NJ) was added to one of the cell smears. The second cell smear on the slide was used as a control and was stained with 25 μl of a bivalent fluorescein isothiocyanate-conjugated mouse monoclonal antibody specific for both herpes simplex virus type 1 (HSV-1) and HSV-2 (Baxter Diagnostics, Inc., Deerfield, Ill.). Both antibody preparations contained an Evans blue counterstain. Appropriate positive VZV and HSV antigen control slides were also stained. The slides were incubated for 30 min at 37°C in a humidified chamber and then washed for 5 min in phosphate-

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TABLE 1. Comparison of DFA and culture for detection of VZV

DFA result	No. of culture results		
	Positive	Negative	Total
Positive	37	40	77
Negative	2	61	63
Total	39	101	140

buffered saline, pH 7.4, and allowed to air dry. The slides were mounted with coverslips in buffered glycerol and examined with a Leitz Laborlux S (Leica, Inc., Malvern, Pa.) epifluorescence microscope at a magnification of ×400. Specimens exhibiting at least 25 intact epithelial cells were considered adequate for cellular content, and a typical apple-green fluorescence in the cytoplasm of two or more cells in the entire smear was considered positive for VZV. All positive specimens were blindly confirmed by a second member of the laboratory staff.

All swabs for viral isolation were received and processed by the laboratory within 1 to 4 h of collection. Tubes, one each, of human diploid lung fibroblasts (MRC-5; American Type Culture Collection [ATCC; Rockville, Md.,] CCL 171), human lung carcinoma cells (A549; ATCC CCL 185), and primary rhesus monkey kidney (PRMK) cells were inoculated with 0.2 ml of clinical specimen. If the physician obtaining the specimen indicated a suspicion of possible HSV infection, a tube of mink lung cells (ATCC CCL 64) was also inoculated. A549, MRC-5 and mink lung cells were propagated in the laboratory and used within 7 days of confluency. PRMK cells were purchased (BioWhittaker, Walkersville, Md.) and used within 1 week of receipt. Inoculated tubes were incubated in slanted stationary racks at 37°C and examined daily for characteristic VZV cytopathic effects (CPE) for the first 7 days and then every other day for a minimum of 21 days. Suspected VZV isolates were confirmed by direct immunofluorescence, using the Ortho Diagnostic Systems fluorescein-conjugated mouse monoclonal antibody 3B3. All CPE and positive immunofluorescence typical for VZV were routinely verified by two members of the laboratory staff. Laboratory personnel who processed virus cultures for VZV were blinded to all previous DFA results.

When DFA and viral culture results were discrepant, a retrospective review of patient medical records was accomplished to determine if a clinical diagnosis of VZV infection had been made. A clinical diagnosis of primary VZV infection was confirmed if the patient had no prior history of infection and had vesicular lesions which were disseminated and pruritic or painful. A clinical diagnosis of herpes zoster was confirmed if the patient had a prior history of infection or was previously known to have a positive VZV serology and had painful vesicular lesions which were in a dermatomal distribution.

A total of 79 (56%) specimens were positive for VZV by either DFA or viral culture (Table 1). Seventy-seven (97.5%) specimens were detected by DFA, while only 39 (49.4%) were positive by culture. Forty (51%) specimens were positive by DFA alone, 2 (3%) were positive by culture only, and 37 (47%) were positive by both culture and DFA. No evidence of VZV was detected by either method in specimens from 61 patients considered on clinical grounds to have a low suspicion of VZV infection. Discordant results between the two methods were observed for 42 specimens. Of the discordant specimens, 40 were positive by DFA with no evidence of VZV in viral culture. VZV was isolated from two specimens that were negative by DFA. All specimens examined for VZV were negative for HSV by DFA, and there was no laboratory evidence of HSV or other viral infections identified by conventional cell culture. Of interest is that with proper instruction and continued education for specimen collection and slide preparation, 88% of the DFA slides submitted during the time of this study had adequate cell numbers to allow their inclusion in the study.

Medical records were available for review from 37 (88.1%) of the 42 patients with discordant results. Sixteen of the patients had an underlying diagnosis of hematological malignancy, six were infected with human immunodeficiency virus, three were transplant recipients, one was a neonate, one had a primary immunodeficiency, five were normal hosts, and five had miscellaneous diagnoses, including muscular dystrophy, Guillain-Barré syndrome, chronic renal failure, inflammatory bowel disease, and β-thalassemia. A clinical diagnosis of primary varicella was confirmed for 20 patients, while 17 patients had documented clinical evidence of herpes zoster. Considering that all specimens which yielded a positive result by either DFA or viral culture were from patients with VZV infection, the sensitivity and negative predictive values, respectively, were 97.5% (77 of 79) and 96.8% (61 of 63) for DFA and 49.4% (39 of 79) and 60.4% (61 of 101) for viral culture. Both DFA and culture proved to be 100% (61 of 61 specimens) specific when clinical diagnosis was used as the gold standard. If the five specimens with discordant results (all DFA positive and culture negative) and unconfirmed clinical VZV infection were assumed to be falsely positive by DFA, the sensitivity, specificity, and positive and negative predictive values of DFA would be 97.3% (72 of 74), 92.4% (61 of 66), 93.5% (72 of 77), and 96.8% (61 of 63), respectively. Among patients with discrepant test results, 10 of 37 (27%) with verified VZV infection had been treated with acyclovir before a specimen was obtained. Eight of these patients, including the two with positive viral cultures and negative DFA, were on therapy for ≤24 h, one received therapy for ≤48 h, and one had been given intermittent acyclovir for 1 week.

Analysis of results for the 39 specimens with positive viral cultures revealed that VZV was isolated most frequently from A549 cells (38 of 39) and then from the PRMK (23 of 39) and MRC-5 (16 of 39) cell lines (Table 2). The mean times to detection of virus CPE were 11 days for both A549 and PRMK cells and 14 days for the MRC-5 cells, although this difference was not statistically significant (*t*-test). A total of 32 (82.1%) VZV isolates were detected first in A549 cells compared with 12 (30.8%) and 8 (20.5%), respectively in PRMK and MRC-5 cells. Nine (23.1%) VZV isolates were only positive in A549 cells, while one (2.6%) isolate was detected only in MRC-5 cells. No VZV isolates were found growing in PRMK cells alone. The CPE for VZV in A549 cells was quite distinct from the unaltered monolayer and readily apparent on microscopic examination (Fig. 1). It was characteristically focal and the initial lesions appeared as discrete plaques of irregularly rounded, swollen, refractile cells which progressed slowly in

TABLE 2. Growth of clinical isolates of VZV in different cell lines

Cell line	Growth of VZV isolates			
	No. (%) positive	No. detected first in given cell line	No. only positive in given cell line	Mean time to positivity (days)[a]
MRC-5	16 (41)	8	1	14.6 ± 6.9
PRMK	23 (59)	12	0	11.6 ± 5.5
A549	38 (97)	32	9	11.3 ± 4.7

[a] Mean time to positivity among the three cell lines was not statistically significant (*t* test).

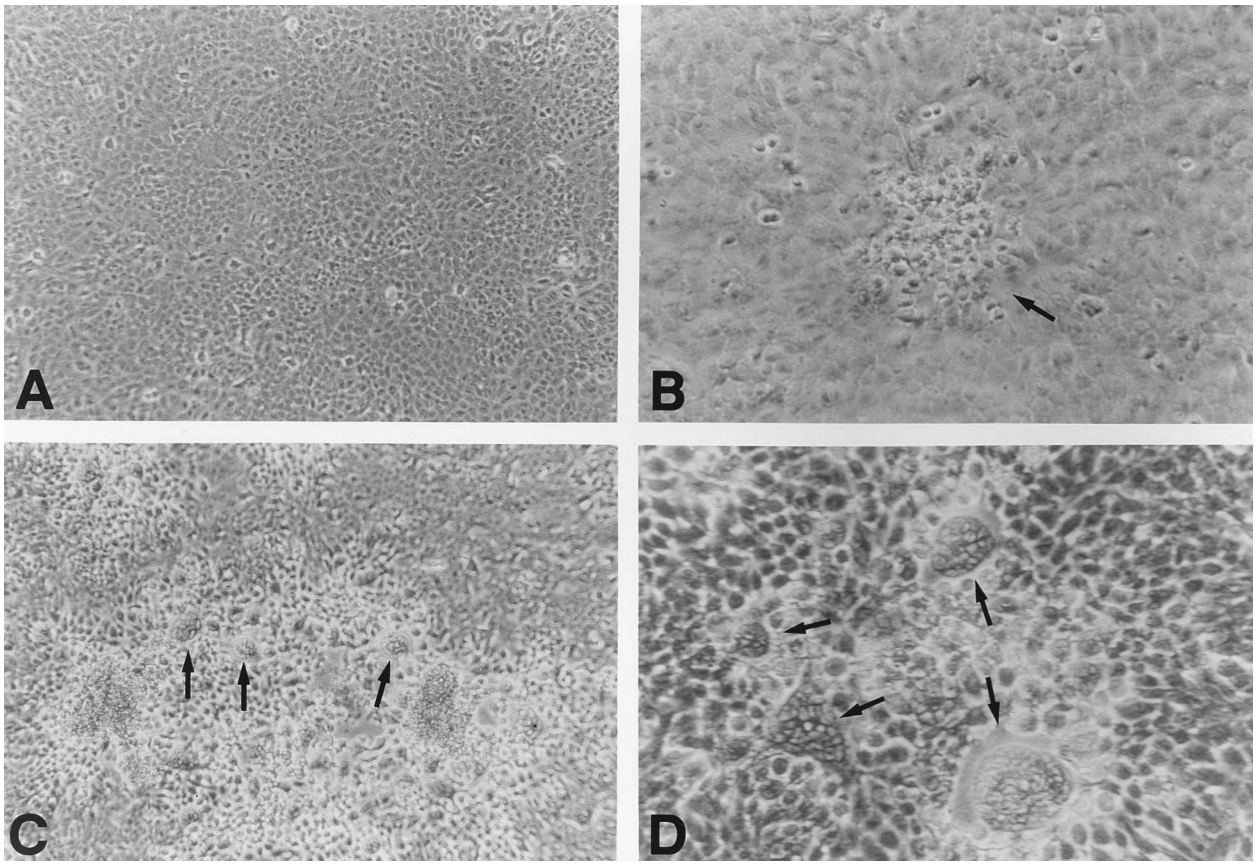

FIG. 1. CPE for VZV in monolayers of A549 cells. (A) Uninfected monolayer of A549 epithelial cells (magnification, ×40); (B) early plaque formation (arrow) following VZV infection (magnification, ×100); (C) coalescence of several VZV-infected plaques (magnification, ×40; note numerous multinucleated giant cells [arrows] within the plaques); (D) higher magnification (×100) of the fried-egg appearance (arrows) of the VZV-infected multinucleated giant cells.

size and number over the first 1 to 2 weeks of culture. In many of the plaques, multinucleated giant cells having a fried-egg appearance were observed. Eventually, the individual foci increased in size and coalesced to involve the entire cell monolayer. A similar CPE was observed for VZV growing in PRMK cells. Presumptive identification of VZV in MRC-5 cells was based on observing a focal CPE which appeared as small groups of swollen, granular, refractile cells progressing linearly along the long axis of the fibroblasts. Specific identification of all suspected VZV isolates was confirmed by DFA, using the Ortho 3B3 monoclonal antibody.

The current study provides one of the largest series of positive specimens to be compared prospectively and in parallel by DFA and viral culture for the detection of VZV infection. DFA with a directly conjugated murine monoclonal antibody was found to be significantly more sensitive than viral culture for the laboratory detection of VZV infection in our hospital population. Additionally, the predictive value of a negative test result was much greater for DFA than for viral culture. Overall, DFA provided a rapid (turnaround time of 1 h), simple, and practical method for early diagnosis of VZV. Our results are in agreement with other investigators who have employed VZV-specific polyclonal (6, 13, 18) or monoclonal (9, 14–16) reagents to demonstrate the superior sensitivity of direct immunofluorescent-antibody staining techniques over viral culture.

Some of the discrepancies between DFA and viral culture in our study may be attributed to the administration of antiviral therapy to certain patients. Acyclovir was used prior to specimen collection in 27.0% of cases showing discordant results. The majority of patients, however, had received acyclovir for ≤24 h. Other factors which may influence the sensitivity of virus culture include the need for rapid processing of specimens after collection due to the lability of VZV and the difficulty of culturing virus from lesions that are over 5 days old (18). Although every effort was made to provide optimum conditions for the isolation of VZV, delays of up to 4 h were experienced for the transport and processing of some specimens examined in this study. Eleven specimens from the discordant patient group which were positive by DFA but negative by culture were collected from lesions that were greater than 4 days old (eight specimens were greater than 5 days old). Interestingly, the lesions of both patients found to have VZV by culture but not by DFA were less than 1 day old, possibly suggesting a lower number of cells expressing viral antigens in these new lesions.

In most laboratories, human diploid fibroblasts, such as MRC-5 and WI-38 cells, are employed as the cell lines of choice for the isolation of VZV. With the addition of A549 and PRMK cells to our culture protocol for VZV, we were able to detect a significant number of isolates that did not produce CPE in fibroblast cells. To our knowledge, this is the first description of the utility of A549 cells to grow VZV from clinical specimens. These cells successfully grew VZV more often than either PRMK or MRC-5 cells, and the CPE was easily recognized.

In conclusion, this study confirms the utility of direct immunofluorescence for the rapid and accurate detection of VZV from lesion scrapings. The procedure is easy to perform and is well suited for use in the routine diagnostic laboratory. Additionally, A549 cells appear to provide enhanced detection of VZV in cell culture, although the reported sensitivity and specificity of DFA would obviate the need to routinely perform cultures in parallel for this virus.

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REFERENCES

1. **Arvin, A. M., and C. M. Koropchak.** 1980. Immunoglobulins M and G to varicella-zoster virus measured by solid phase radioimmunoassay: antibody responses to varicella and herpes zoster infections. J. Clin. Microbiol. **12:** 367–374.
2. **Balfour, H. H., Jr.** 1984. Intravenous acyclovir therapy for varicella in immunocompromised children. J. Pediatr. **104:**134–136.
3. **Balfour, H. H., Jr.** 1991. Varicella-zoster virus infections in the immunocompromised host. Natural history and treatment. Scand. J. Infect. Dis. **78**(Suppl.)**:**69–74.
4. **Balfour, H. H., Jr., B. Bean, O. L. Laskin, R. F. Ambinder, J. D. Meyers, J. C. Wade, J. A. Zaia, D. Aeppli, L. E. Kirk, A. C. Segreti, R. E. Keeney, and Burroughs Wellcome Collaborative Acyclovir Study Group.** 1983. Acyclovir halts progression of herpes zoster in immunocompromised patients. N. Engl. J. Med. **308:**1448–1453.
5. **Cohen, P. R., V. P. Beltrani, and M. E. Grossman.** 1988. Disseminated herpes zoster in patients with human immunodeficiency virus infection. Am. J. Med. **84:**1076–1080.
6. **Drew, W. L., and L. Mintz.** 1980. Rapid diagnosis of varicella-zoster virus infection by direct immunofluorescence. Am. J. Clin. Pathol. **73:**699–701.
7. **Feldman, S., and L. Lott.** 1987. Varicella in children with cancer: impact of antiviral therapy and prophylaxis. Pediatrics **80:**465–472.
8. **Folkers, E., J. Vreeswijk, A. P. Oranje, and J. N. Duivenvoorden.** 1989. Rapid diagnosis in varicella and herpes zoster: re-evaluation of direct smear (Tzanck test) and electron microscopy including colloidal gold immunoelectron microscopy in comparison with virus isolation. Br. J. Dermatol. **121:**287–296.
9. **Gleaves, C. A., C. F. Lee, C. I. Bustamante, and J. D. Meyers.** 1988. Use of murine monoclonal antibodies for laboratory diagnosis of varicella-zoster virus infection. J. Clin. Microbiol. **26:**1623–1625.
10. **Jura, E., E. G. Chadwick, S. H. Josephs, S. P. Steinberg, R. Yogev, A. A. Gershon, K. M. Krasinski, and W. Borkowsky.** 1989. Varicella-zoster virus infections in children infected with human immunodeficiency virus. Pediatr. Infect. Dis. J. **8:**586–590.
11. **Kido, S., T. Ozaki, H. Asada, K. Higashi, K. Kondo, Y. Hayakawa, T. Morishima, M. Takahashi, and K. Yamanishi.** 1991. Detection of varicella-zoster virus (VZV) DNA in clinical samples from patients with VZV by the polymerase chain reaction. J. Clin. Microbiol. **29:**76–79.
12. **Novelli, V. M., P. A. Brunell, C. F. Geiser, S. Narkewicz, and L. Frierson.** 1988. Herpes zoster in children with acute lymphocytic leukemia. Am. J. Dis. Child. **142:**71–72.
13. **Olding-Stenkvist, E., and M. Grandien.** 1976. Early diagnosis of virus-caused vesicular rashes by immunofluorescence on skin biopsy. Scand. J. Infect. Dis. **8:**27–35.
14. **Perez, J. L., A. Garcia, J. Niubo, J. Salva, D. Podzamczer, and R. Martin.** 1994. Comparison of techniques and evaluation of three commercial monoclonal antibodies for laboratory diagnosis of varicella-zoster virus in mucocutaneous specimens. J. Clin. Microbiol. **32:**1610–1613.
15. **Rawlinson, W. D., D. E. Dwyer, V. L. Gibbons, and A. L. Cunningham.** 1989. Rapid diagnosis of varicella-zoster virus infection with a monoclonal antibody based direct immunofluorescence technique. J. Virol. Methods **23:**13–18.
16. **Sadick, N. S., P. D. Swenson, R. L. Kaufman, and M. H. Kaplan.** 1987. Comparison of detection of varicella-zoster virus by the Tzank smear, direct immunofluorescence with a monoclonal antibody, and virus isolation. J. Am. Acad. Dermatol. **17:**64–69.
17. **Schirm, J., J. J. M. Meulenberg, G. W. Pastoor, P. C. van Voorst Vader, and F. P. Schroder.** 1989. Rapid detection of varicella-zoster virus in clinical specimens using monoclonal antibodies on shell vials and smears. J. Med. Virol. **28:**1–6.
18. **Schmidt, N. J., D. Gallo, V. Devlin, J. D. Woodie, and R. W. Emmons.** 1980. Direct immunofluorescence staining for detection of herpes simplex and varicella-zoster virus antigens in vesicular lesions and certain tissue specimens. J. Clin. Microbiol. **12:**651–655.
19. **Schuchter, L. M., J. R. Wingard, S. Piantadosi, W. H. Burns, G. W. Santos, and R. Saral.** 1989. Herpes zoster infection after autologous bone marrow transplantation. Blood **74:**1424–1427.
20. **Solomon, A. R., J. E. Rasmussen, and J. S. Weiss.** 1986. A comparison of the Tzanck smear and viral isolation in varicella and herpes zoster. Arch. Dermatol. **122:**282–285.
21. **Vreeswijk, J., E. Folkers, F. Wagenaar, and J. G. Kapsenberg.** 1988. The use of colloidal gold immunoelectron microscopy to diagnose varicella-zoster virus (VZV) infections by rapid discrimination between VZV, HSV-1 and HSV-2. J. Virol. Methods **22:**255–271.
22. **Weller, T. H.** 1983. Varicella and herpes zoster. II. Changing concepts of the natural history, control, and importance of a not-so-benign virus. N. Engl. J. Med. **309:**1434–1440.
23. **West, P. G., B. Aldrich, R. Hartwig, and G. J. Haller.** 1988. Increased detection rate for varicella-zoster virus with combination of two techniques. J. Clin. Microbiol. **26:**2680–2681.
24. **Williams, M. G., J. D. Almeida, and A. F. Howatson.** 1962. Electron microscopy studies on viral skin lesions: a simple and rapid method of identifying virus particles. Arch. Dermatol. **86:**290–297.