JCM
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Should Varicella-Zoster Virus Culture Be Eliminated? A Comparison of Direct Immunofluorescence Antigen Detection, Culture, and PCR, with a Historical Review

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A comparison of direct fluorescent-antibody assay (DFA), culture, and two PCR assays disclosed sensitivities of 87.8%, 46.3%, and 97.6% and 100%, respectively. We reviewed 1,150 results for clinical specimens submitted for DFA and culture and found that only 17 were culture positive/DFA negative. The incremental cost to detect these 17 positives was $3,078/specimen.

Primary varicella-zoster virus (VZV) infection in children produces chickenpox, which is a self-limited disease; in adults, primary VZV infection produces more severe disease that may include viral pneumonia (5, 8). The VZV, like other members of the herpesviridae, produces a latent infection. The reactivation of latent virus produces shingles, which occurs most commonly in the elderly, the immunosuppressed, and individuals with other diseases (8). This manifestation is often obvious upon physical examination, as the vesicular lesions have a dermatomal distribution (7). Atypical presentations, however, may occur; occasionally, VZV may produce meningoencephalitis and/or disseminated disease (4, 7).

When the clinical impression of either primary or reactivation disease requires confirmation, diagnostic microbiologic studies are needed. Traditional virology tests for VZV include the direct microscopic assessment of the specimen following staining with a fluorescently labeled antibody directed against VZV (i.e., direct fluorescent-antibody assay [DFA]) and/or viral culture using either a shell vial or, less commonly, a tube culture (1, 2, 6). A molecular approach to the detection of VZV utilizes VZV-specific rapid-cycle PCR; several authors have demonstrated the feasibility and potential clinical utility of rapid-cycle PCR for the direct detection of VZV (3, 9). We sought to compare the traditional assays, DFA and culture, that are routinely used in our laboratory with two PCR assays, to determine the performance of each of these assays on routinely collected clinical specimens submitted for VZV DFA and culture. Specimen types studied included prominently cutaneous lesions, usually characterized as vesicles, as well as specimens from the eye (i.e., cornea), ear (i.e., external ear), and mouth. The skin lesions were from various anatomic sites, but common among these were lesions from the arm, buttocks, and scalp. The specimens were submitted in viral transport medium (M4; Remel, Lenexa, KS).

The Light Diagnostics VZV DFA (Light Diagnostics, Temecula, CA) was used for the direct detection of VZV in clinical specimens and tissue cultures in our laboratory. In brief, the fluorescein isothiocyanate (FITC)-labeled monoclonal antibody reagent provided would bind to VZV antigen (glycoprotein gpI and the immediate early antigen), if present. Unbound FITC-labeled monoclonal antibody is removed by washing with phosphate-buffered saline (PBS). The FITC-labeled cells demonstrate an apple-green fluorescence when illuminated by UV light, which allows for visualization of the antigen-antibody complex using fluorescence microscopy. The presence of cellular fluorescence indicates a positive specimen. Uninfected cells stain a dull red, due to the presence of Evans blue in the FITC-labeled monoclonal antibody mixture.

For VZV culture, supernatant from an aliquot of M4 (Remel) was inoculated into two shell vials containing the MRC-5 cell line (Diagnostics Hybrids, a Quidel Company, Athens, Ohio) and incubated at 37°C in 5% CO_2. The monolayer of MRC5 was stained with monoclonal conjugate specific for VZV (Light Diagnostics, Millipore, Temecula, CA) at day 2 and day 4. The slide was examined using fluorescence microscopy, as described.

Occasionally, viral cultures submitted for VZV may be overgrown by HSV. This is a limitation of culture, because VZV is the slower replicating of the two viruses and cannot be detected in the presence of the more rapidly replicating HSV. Clinical samples included in this study had both DFA and shell vial culture results reported as either positive or negative for VZV. Samples which were overgrown with HSV were excluded in the comparison of DFA, culture, and PCR, so as to not bias the results against culture.

The first PCR method was performed using the Roche LightCycler (LC PCR). Four hundred microliters of specimen-containing M4 medium was extracted on the easyMag (Nuclisens, bioMérieux, Durham, NC) according to the manufacturer's instructions. One VZV-specific PCR assay was performed on the LightCycler system, as previously described. The primers and fluorescence-resonance energy transfer probes used with this assay were as follows: forward primer, 5′-GAC AAT ATC ATATAC ATG GAATGT G-3′; reverse primer, 5′-GCG GTA GTA ACA GAG AAT TTC TT-3′; hybridization probe-1, 5′-CGA AAA TCC

Received 20 December 2011 Returned for modification 18 January 2012 Accepted 24 September 2012
Published ahead of print 3 October 2012
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doi:10.1128/JCM.06753-11

TABLE 1 Characterization of each specimen by the various assays compared with the consensus assignment of each specimen

Test	No. of specimens	
	Consensus positive ($n = 41$)	Consensus negative ($n = 32$)
VZV DFA		
Positive	36	2
Negative	5	30
VZV culture		
Positive	19	0
Negative	22	32
LightCycler VZV PCR		
Positive	40	0
Negative	1	32
RealStar VZV PCR		
Positive	41	3
Negative	0	29

AGA ATC GGA ACT TCT T-FITC-3′; and hybridization probe-2, 5′-LC640-CCA TTA CAG TAA ACT TTA GGC GGT C-3′. The LightCycler PCR protocol consisted of 10 min at 95°C for *Taq* polymerase activation, 45 cycles of PCR amplification (95°C for 10 s, 60°C for 10 s, and 72°C for 20 s), melting (40 to 95°C at 0.1°C/s), and a cooling step (40°C for 30 s).

The RealStar VZV PCR kit (Altona Diagnostics, Hamburg, Germany) was also used to detect VZV by rapid-cycle PCR. This assay was performed on the ABI Prism 7500 SDS (Applied Biosystems, Carlsbad, CA). The PCR protocol consisted of 10 min at 95°C and 45 cycles of amplification (95°C for 15 min, 58°C for 1 min). Amplified product was detected in the FAM (6-carboxy-fluorescein) channel, and the internal control included with this assay was detected in the JOE (5′-dichloro-dimethoxy-fluorescein) channel. The sequences of the primers and probe used in this assay are proprietary.

Seventy-three specimens were assessed by DFA, culture, and both PCR assays, as described above. Specimens were considered to contain VZV if ≥2 tests were positive. Forty-one (out of 73; 56%) of the specimens contained VZV by this criteria. The ability of each assay to appropriately characterize each clinical specimen is shown in Table 1. The VZV culture yielded the most false-negative results. The sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV), respectively, with 95% exact binomial confidence intervals in parentheses, were as follows: DFA, 87.8% (74% to 96%), 93.8% (79% to 99%), 94.7% (82% to 99%), and 85.7% (70% to 95%); culture, 46.3% (31% to 63%), 100% (99% to 100%), 100% (82% to 100%), and 59.3% (45% to 72%); LC PCR, 97.6% (87% to 100%), 100% (89% to 100%), 100% (91% to 100%), and 97.0% (84% to 100%); and RealStar VZV PCR kit PCR, 100% (91% to 100%), 90.6% (75% to 98%), 93.2% (81% to 99%), 100% (88% to 100%).

The pairwise test-to-test comparison, based on McNemar's exact test, for each of the assays studied demonstrated the following. There was a statistically significant difference between culture and DFA ($P < 0.01$), culture and LC PCR ($P < 0.01$), and culture and RealStar VZV PCR ($P < 0.01$). There was no statistically significant difference between DFA and either the LC PCR ($P = 0.73$) or the RealStar VZVPCR ($P = 0.11$). Finally, there was no statistically significant difference between the two PCR assays studied ($P = 0.13$).

We performed a review of the electronic medical records from November 2001 through July 2011 to identify specimens for which both VZV DFA and VZV culture were ordered. This review disclosed 1,150 specimens. Of these, 163 (14.2%) were unable to be completed due to HSV overgrowth (140; 12.2% of total), contamination (16; 1.4% of total), or toxicity (7; 0.61% of total).

Of the remaining 987 specimens that could be fully examined, 199 (20.2%) were positive by both methods, whereas 616 (62.4%) were negative by both methods. It is not possible in a retrospective review to definitively determine false-positive and false-negative rates. However, given the high specificity of both the VZV DFA and the VZV culture, we believe it is valid to consider positives from either of these assays as likely to represent true positives. There were 371 (37.6%) of the 987 specimens that were positive by one of the two methods; these are considered here as true positives (i.e., the specimen contained VZV). Of the 371 VZV-containing specimens, 155 (41.2%) were mischaracterized as negative by culture, whereas only 17 (4.6%) were mischaracterized as negative by DFA. We used our costing and timing information associated with this test at our institution to determine the cost of incremental positives that were detected by VZV culture that were not detected by VZV DFA. Of the 1,150 historical specimens submitted for DFA and culture, only 17 were culture positive/DFA negative. The cost of the VZV culture at our institution was $45.53 per culture (labor, 38 min × $0.48/minute = $18.24; materials, $27.29). Therefore, $52,359.50 ($45.53/culture × 1,150 cultures) was spent for VZV cultures in this review. The incremental cost expended to detect the 17 VZV-containing specimens that were not detected by VZV DFA was $3,080/specimen ($52,359.50/17 culture-positive, DFA-negative specimens).

The laboratory-based confirmation of the suspected clinical diagnosis of a VZV infection is important to confirm the etiologic agent of disease. False negatives are problematic, since the physician may be led to believe that a particular etiologic agent has been excluded and, therefore, may order additional, unnecessary tests. In this study, an astounding 22 of 41 specimens (53.7%), which were confirmed to contain VZV by at least two other testing methods, were falsely negative by culture; the sensitivity of culture was a dismal 46.3%. Although culture was found to be highly specific, in the assay comparison portion of this study, every specimen that demonstrated VZV via culture had an associated positive VZV DFA (data not shown). Therefore, for the 73 specimens tested by four methods, culture added nothing to the detection of VZV that was not already known through the use of VZV-specific DFA. In contrast, the VZV DFA missed five of the 41 consensus-positive specimens (12.2%), yielding a still respectable sensitivity of 87.8%. Two of the 32 consensus-negative specimens (6.3%) were falsely called positive by DFA, which produced a specificity of 93.8%.

Two PCR assays for VZV were evaluated in this study. The LightCycler assay was obtained following literature review and used as described. The limitation of this assay was the lack of an internal amplification control. This assay appropriately characterized all but one sample with respect to the presence or absence of VZV. This assay did not detect one of the 41 consensus specimens (2.4%), which lowered the sensitivity of this assay to 97.6%. The RealStar VZV PCR assay detected all the specimens that contained

VZV (i.e., 100% sensitivity). There were three of the 32 consensus-negative specimens (9.4%) that were falsely positive by the RealStar VZV PCR assay, which produced a specificity of 90.6%; all of these PCRs had late crossing thresholds. None of the specimens were inhibited, as determined by the internal control included with the RealStar VZV PCR assay.

The findings of this study support the continued use of VZV-specific DFA as a rapid, sensitive, and specific test for the detection of this virus. In contrast, it raises the question regarding the added value of cell culture for the detection of VZV in a clinical diagnostic algorithm. Our historical review demonstrated that a significant percentage of VZV culture cannot be completed due to toxicity, contamination, or HSV overgrowth. In addition, it confirmed that most VZV-positive cultures were associated with positive VZV DFA results. The cost associated with the detection of the 17 additional positives in the 1,150 specimen tests was unacceptably high, at $3,050/incremental positive.

Our study confirms that a PCR-based approach to the detection of VZV in clinical specimens obtained from vesicular lesions is the most sensitive and specific. This is similar to the results of other investigators who have reported that rapid-cycle PCR is both a highly sensitive and specific method for the detection of VZV (3, 9). PCR-based diagnostics, however, are more complicated to perform and are more costly than DFA. An algorithm for such specimens may include an initial VZV DFA, with a VZV-specific PCR used for the rare instances in which the DFA is negative but the clinical suspicion of VZV remains high.

REFERENCES

1. **Chan EL, Brandt K, Horsman GB.** 2001. Comparison of Chemicon Simul-Fluor 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. Clin. Diagn. Lab. Immunol. **8**:909–912.
2. **Coffin SE, Hodinka RL.** 1995. Utility of direct immunofluorescence and virus culture for detection of varicella-zoster virus in skin lesions. J. Clin. Microbiol. **33**:2792–2795.
3. **Espy MJ, et al.** 2000. Diagnosis of varicella-zoster virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. **38**:3187–3189.
4. **Flamholc L.** 1996. Neurological complications in herpes zoster. Scand. J. Infect. Dis. Suppl. **100**:35–40.
5. **Hambleton S.** 2005. Chickenpox. Curr. Opin. Infect. Dis. **18**:235–240.
6. **Huang YT, Hite S, Duane V, Yan H.** 2002. CV-1 and MRC-5 mixed cells for simultaneous detection of herpes simplex viruses and varicella zoster virus in skin lesions. J. Clin. Virol. **24**:37–43.
7. **Strommen GL, Pucino F, Tight RR, Beck CL.** 1988. Human infection with herpes zoster: etiology, pathophysiology, diagnosis, clinical course, and treatment. Pharmacotherapy **8**:52–68.
8. **Weaver BA.** 2009. Herpes zoster overview: natural history and incidence. J. Am. Osteopath. Assoc. **109**:S2–S6.
9. **Weidmann M, Meyer-Konig U, Hufert FT.** 2003. Rapid detection of herpes simplex virus and varicella-zoster virus infections by real-time PCR. J. Clin. Microbiol. **41**:1565–1568.