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Diagnosis of Varicella-Zoster Virus Infections in the Clinical Laboratory by LightCycler PCR

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Varicella-zoster virus (VZV) causes vesicular dermal lesions which are clinically evident as varicella (primary infection) or zoster (reactivated) diseases. The LightCycler system (Roche Molecular Biochemicals) is a newly developed commercially available system designed to rapidly perform PCR with real-time detection of PCR products using a fluorescence resonance energy transfer. We compared the detection of VZV from dermal specimens by shell vial cell culture (MRC-5) and by LightCycler PCR. Of 253 specimens, VZV was detected in 23 (9.1%) by shell vial cell cultures and 44 (17.4%) by LightCycler PCR directed to a nucleic acid target sequence in gene 28. Twenty-one of 44 (47.7%) specimens were exclusively positive by LightCycler PCR; the shell vial cell culture assay was never positive when DNA amplification was negative (specificity, 100%). VZV DNA was detected in 39 of 44 (88.6%) specimens positive during cycles 10 through 30 of the LightCycler PCR. These VZV DNA-positive specimens (cycles 10 to 30) and 5 of 11 other PCR positive specimens (cycles 31 to 36) were confirmed by another LightCycler PCR directed to another (gene 29) target of the viral genome. For routine laboratory practice, all specimens yielding amplified DNA to the VZV gene 28 target can be considered positive results. The increased sensitivity (91%) of the LightCycler PCR for detection of VZV, rapid turnaround time for reporting results, virtual elimination of amplicon carryover contamination, and equivalent costs compared to shell vial cell culture for detection of VZV indicate the need for implementation of this technology for routine laboratory diagnosis of this viral infection.

The most common dermal manifestation resulting from primary infection with varicella-zoster virus (VZV) is chickenpox, generally occurring in early childhood. Reactivation of latent virus occurs in about 10 to 20% of adults and produces vesicles that are typically confined to a single dermatome of the skin (16). VZV infections can cause systemic infections of the central nervous and respiratory systems in immunologically healthy patients and produce disseminated disease of multiple organ systems in those with impaired immunologic defenses (6). Laboratory diagnosis is important for distinguishing herpes simplex virus (HSV) from VZV infections, since the clinical presentation of zoster can be confused with the dermal distribution produced by HSV (10).

Standard laboratory diagnosis has been obtained by culture of the virus in diploid fibroblasts seeded into shell vial cell cultures or directly by immunostaining of viral antigens in infected cells collected by swabs of vesicles from patients (2, 3, 8, 13). In the past, several studies have demonstrated the superiority of detection of VZV antigens by immunologic techniques or, more recently, by molecular amplification of viral DNA by PCR assays compared with serologic assays for immunoglobulin G or A class antibodies or the cultivation of this virus in cell cultures (1, 4, 9, 15). Sauerbrei et al. reported that the laboratory diagnoses of VZV infections of 100 patients by culture (20%), antigen detection by immunofluorescence (82%), and serology (48%) were inferior relative to those by PCR (95%) (11).

We recently reported enhanced sensitivity and rapid detection of HSV DNA by LightCycler PCR using genital and dermal specimens of patients compared with shell vial cell culture techniques. In our laboratory, we process each dermal specimen, in contrast to genital specimens, for the detection of both HSV and VZV infections. Our goal in the present study was to optimize the primers, probes, and conditions for the detection of VZV by LightCycler PCR and compare the results of this assay with those of shell vial cell culture isolation of the virus from dermal specimens. We found a 91% increase in detection of VZV by a LightCycler PCR from dermal specimens, which indicates the need for implementation of this assay for replacement of cell culture assays for diagnosis of this viral infection.

MATERIALS AND METHODS

Specimens and cell cultures. Dermal swabs ($n = 253$) from patients suspected of having VZV infections were extracted and inoculated into MRC-5 shell vial cell cultures as previously described for HSV (7).

Nucleic acid extraction. Nucleic acids were extracted (IsoQuick; Orca Research, Inc., Bothell, Wash.) and amplified by LightCycler PCR (5).

LightCycler PCR. The LightCycler instrument (Roche Molecular Biochemicals) amplifies (in 30 to 40 min) target nucleic acid and monitors the development of PCR product by fluorescence assay after each cycle (denaturation, annealing, and extension). PCR primers for detection of target DNA in gene 28 were as follows: sense, 5′-GAC AAT ATC ATA TAC ATG GAA TGT G-3′; antisense, 5′-GCG GTA GTA ACA GAG AAT TTC TT-3′. Probes used were 5′-CGA AAA TCC AGA ATC GGA ACT TCT T-fluorescein-3′ and 5′-Red 640-CCA TTA CAG TAA ACT TTA GGC GGT C phosphate-3′ directed to a target of the 282-bp product (11). The master mix was optimized for the VZV, gene 28 LightCycler assay by eliminating dimethyl sulfoxide and by using 4 mM MgCl and 1 μM primers.

All samples were amplified by LightCycler PCR with primers directed to two genes of the virus: gene 28 and gene 29. Primers and probe for detection of VZV DNA gene 29 (single-stranded DNA binding protein and gene 28) were designed using the Oligo software (Molecular Biology Insights, Inc., Cascade, Colo.): sense, 5′-TGT CCT AGA GGA GGT TTT ATC TG-3′; antisense, 5′-CAT CGT CTG TAA AGAC TTA ACC AG-3′. Probes directed to target VZV DNA were of the 202-bp product. Both sets of hybridization probes contain a donor fluorophore (fluorescein) on the 3′ end and 5′-GGG AAA TCG AGA AAC CAC CCT ATC GGA C-3′, which, when excited by an external light source, emits light that was absorbed by a second hybridization probe, 5′-Red 640-AA GTT CGC GGT ATA ATT GTC AGT GGC G-phosphate-3′ with an acceptor fluorophore, Red 640, at the 5′ end. Both assays detected ≥20 genomic copies of VZV.

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TABLE 1. Detection of VZV DNA by LightCycler PCR and by shell vial cell culture

Cycle no.	No. of specimens positive by:		
	LightCycler with the following target:		Shell vial cell culture
	Gene 28	Gene 29	
0–30	39	39	23
31	0	1	0
32	0	0	0
33	1	2	0
34	3	4	0
35	1	3	0
36	0	1	0
Total	44	50	23

Specimen volumes, master mix composition, and amplification protocol for VZV DNA were as previously described for HSV DNA except for the use of 4 mM MgCl, 1 μM primers (gene 29), and 3% dimethyl sulfoxide (5).

RESULTS

Of 253 dermal specimens, VZV was detected in 23 (9.1%) by shell vial cell cultures and 44 (17.4%) (gene 28) and 50 (19.7%) (gene 29) by LightCycler PCR tests (Table 1). Twenty-one of 44 (47.7%) (gene 28) and 27 of 50 (54.0%) (gene 29) specimens were exclusively positive by LightCycler PCR; the shell vial cell culture assay was never positive when DNA amplification was negative (specificity, 100%). VZV DNA was detected in 39 of the 44 (88.6%) (gene 28) and 39 of 50 (78.0%) (gene 29) total specimens positive during cycles 10 through 30 by the LightCycler assay. In addition, of the 23 total specimens positive by the shell vial assay, VZV DNA was detected by both LightCycler assays in these samples by cycle 26, indicating a direct relationship between the capability of culturing the virus by the shell vial assay and the recognition of amplified product in the early cycles of LightCycler PCR.

Of the total 50 specimens detected by LightCycler PCR (gene 29), 11 samples were detected between cycles 30 and 36; 6 fewer samples ($n = 44$) were detected by LightCycler PCR (gene 28). VZV DNA was never exclusively detected by gene 28 in the absence of a positive LightCycler result using the gene 29 assay. Specificity of the LightCycler assay was further demonstrated by melting point analysis, which was performed with all samples from which a fluorescent signal was generated. All positive samples had a melting curve consistent with the positive VZV control.

DISCUSSION

HSV (dermal and/or genital) and VZV (dermal) are the only two viruses routinely recovered from these sources in the clinical laboratory (14). Together (HSV, 71%; VZV, 6%), these two herpesviruses represent 77% of the almost 20,000 viruses detected in cell cultures in our laboratory over a 5-year period of time (1994 to 1998).

We previously reported a 22% increase in the sensitivity of LightCycler PCR compared to cell culture for detection of HSV DNA from clinical samples (5). We currently have demonstrated a 1.9-fold increase (91%) in the detection of VZV DNA by LightCycler PCR ($n = 44$ [gene 28]) in dermal specimens compared to shell vial cell culture ($n = 23$). These results were not unexpected, since numerous reports over the last 20 years have documented the difficulty of recovering VZV in tube and shell vial cell cultures relative to other diagnostic methods, particularly antigen detection by immunofluorescence and conventional PCR methods (1, 3, 12).

Further, we have demonstrated the specificity of the LightCycler VZV assay in that VZV-specific primers and probes amplified only VZV DNA. DNA from HSV, cytomegalovirus, Epstein-Barr virus, and human herpesviruses 6, 7, and 8 tested with VZV-specific primers and probes were uniformly negative. In addition, of a total of 500 dermal, genital, and ocular samples tested by LightCycler PCR, 197 and 44 were confirmed as HSV DNA positive samples and VZV DNA-confirmed samples by another LightCycler PCR. There was no cross-reaction in fluorescence signal between the VZV- and HSV-positive sample.

Results of LightCycler PCR compared to shell vial cell culture were remarkably similar for the laboratory diagnosis of VZV in the present study and for HSV in a previous report (5). In both of these studies, target nucleic acids for HSV (158 of 225 [70.2%]) and VZV (39 of 44 [genes 28 and 29] [88.6%]) were detected by two independent LightCycler PCRs during the first 30 cycles of amplification. In the present report, primers directed to VZV (gene 29) detected 50 specimens with specific viral DNA, whereas 44 specimens were positive for the gene 28 target. Therefore, in routine laboratory practice, we recommend the implementation of primers directed to gene 28 based on a recent publication in which a chemiluminescence assay was used to detect VZV DNA PCR products (11). Even though gene 29 target was more sensitive than that of gene 28 in our study and 44 samples yielding VZV product were positive by PCR directed against both targets, we feel that this conservative approach (positive by both gene targets) should be used as a criterion for reporting results for clinical implementation of the assay.

Negative controls did not generate a product regardless of the number of PCR cycles. Overall, the closed system of LightCycler PCR, which practically eliminates carryover amplification contamination, together with the real-time monitoring of PCR products (especially those detected during the first 30 cycles), and melting curve analysis provides additional confidence of true-positive results compared with conventional gel and Southern blot analysis of PCR amplicons, which do not have these features. In addition, the LightCycler PCR should allow consistent and standardized analysis of clinical samples for the detection of VZV DNA compared to "home brew" assays that have multiple variables incorporated into the assay. Such standardization should become even more achievable with the potential use of an automated nucleic acid extraction instrument (MagNa Pure LC; Roche Molecular Biochemicals) prior to LightCycler PCR amplification. Lastly, the equivalent cost analysis for LightCycler PCR and vastly increased sensitivity compared with shell vial cell culture methods indicate the need for routine implementation of this new diagnostic technique.

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