Clinical Validation of the Lyra Direct HSV 1+2/VZV Assay for Simultaneous Detection and Differentiation of Three Herpesviruses in Cutaneous and Mucocutaneous Lesions

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We evaluated the Lyra Direct HSV 1+2/VZV multiplex real-time PCR assay for the detection and differentiation of herpes simplex virus 1 (HSV-1), HSV-2, and varicella-zoster virus (VZV) on 695 consecutive cutaneous and mucocutaneous lesion specimens. The intra-assay and interassay coefficient of variation values for the Lyra assay were 0.29 to 1.30% and 2.33 to 2.61%, respectively. The sensitivities, specificities, and positive and negative predictive values were 93.4 to 95.0%, 96.1 to 96.8%, 78.0 to 80.3%, and 99.0 to 99.1%, respectively, in comparison to those of viral culture. The values were further improved when a resolution analysis was performed with a laboratory-developed PCR assay.

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2, respectively) and varicella-zoster virus (VZV) cause serious cutaneous and mucocutaneous lesions at every stage in immunocompromised patients (1). Due to the similarities in the clinical presentations of infections caused by these three herpesviruses, a clinical diagnosis needs to be confirmed by laboratory testing to prevent misdiagnosis (2). The need for accurate and specific laboratory diagnostics is especially important in immunocompromised patients who may present with atypical lesions confounding the clinical diagnosis and delaying the institution of appropriate antiviral therapy (1, 3, 4). Consequently, etiologic diagnosis and differentiation of HSV-1, HSV-2, and VZV infections is critical for both patient care and infection control (3, 4).

Traditional laboratory methods for the diagnosis of cutaneous and mucocutaneous HSV-1, HSV-2, and VZV infections include the Tzanck smear, direct fluorescent assay (DFA), and cell culture (including shell vial culture) (3). Several reports have been published on the increased sensitivity of real-time PCR assays for detecting HSV/VZV over the traditional methods described above (5–7). Most published molecular assays utilize separate reactions for either HSV or VZV, and limited multiplex formats for the simultaneous detection and differentiation of HSV-1, HSV-2, and VZV have been reported (7–9). Currently, four molecular assays have been cleared by the Food and Drug Administration (FDA) for the detection and/or typing of HSV-1 and HSV-2 in genital and/or oral lesions: the MultiCode HSV-1&2 assay (Luminex Corporation, Austin, TX), the ProbeTec HSV QX amplified DNA assay (BD Diagnostics, Sparks, MD), the IsoAmp HSV assay (Bio-Helix Corporation, Beverly, MA), and the AmpliVue HSV 1+2 assay (Quidel Corporation, San Diego, CA) (10).

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In May 2014, the FDA cleared the Lyra Direct HSV 1+2/VZV assay (Quidel Corporation, San Diego, CA). This is the first FDA-cleared commercial multiplex real-time PCR assay that simultaneously detects and differentiates HSV-1/2 and VZV DNA in both cutaneous and mucocutaneous lesion specimens. The objectives of this preclinical study were to validate the performance of the

Lyra assay on cutaneous and mucosal specimens, specifically in an immunocompromised oncology patient population.

Clinical samples. Cutaneous or mucocutaneous lesions from symptomatic patients were sampled using a FLOQSwabs (Copan Diagnostics, Inc., Murrieta, CA) and submitted to the laboratory in Remel M4 transport medium (M4RT; Thermo Fisher Scientific, Lenexa, KS) for HSV and/or VZV culture. Six hundred ninety-five consecutive swab specimens were collected between 14 May 2012 and 25 November 2013. Following the completion of the culture setup, the leftover M4RT medium specimens were collected, aliquoted, and stored at -80°C until testing with the Lyra assay and laboratory-developed tests (LDT). The study was granted a waiver of the Health Insurance Portability and Accountability Act (HIPAA) authorization and informed consent (WA0346-13) by the Memorial Sloan-Kettering Cancer Center institutional review board committee.

Tube and shell vial culture. Cell culture was performed by adding 250 μl of M4RT medium to an MRC-5 cell culture tube and an A549 shell vial (Diagnostic Hybrids, Athens, OH), followed by incubation at 37°C for a maximum of 14 days (5, 7, 9). The culture tubes were read every day to detect cytopathic effects. For the shell vial culture, identification was confirmed using fluorescence-labeled monoclonal antibodies against HSV (Bartels; Trinity Biotech, Wicklow, Ireland) and VZV (Merifluor; Meridian Diagnostic, Inc., Cincinnati, OH) at 24 and 48 h after incubation. The slides were read using an Olympus BX40 microscope with a BX-FLA reflected fluorescent light attachment.

Lyra Direct HSV 1+2/VZV assay. The Lyra Direct HSV 1+2/VZV assay uses multiplex real-time PCR to detect and differenti-

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TABLE 1 Intra- and interassay variations of the Lyra Direct HSV-1+2/VZV assay

Virus by variation	Specimen ID ^a	Mean C_T ^b	SD C_T	CV (%) ^c
Intra-assay				
HSV-1	1110	25.89	0.19	0.74
	1141	32.90	0.30	0.90
	1108	29.65	0.15	0.50
HSV-2	1124	18.82	0.08	0.41
	1151	32.21	0.42	1.30
	1120	29.93	0.13	0.44
VZV	1066	17.60	0.10	0.57
	1025	30.82	0.09	0.29
	1028	26.16	0.20	0.75
Interassay				
HSV-1	PC ^d	27.20	0.63	2.33
HSV-2	PC	25.47	0.58	2.26
VZV	PC	22.42	0.58	2.61

^a Specimens used for the intra-assay variations are clinical specimens enrolled in this study. The specimens used for interassay variations were positive controls provided in the kit. ID, identification.

^b C_T , threshold cycle.

^c CV, coefficient of variation.

^d PC, positive control.

ate HSV-1, HSV-2, and VZV DNA in a given specimen. The multiplex real-time PCR is carried out under optimized conditions in a single tube, generating amplicons for HSV-1, HSV-2, VZV, and the process control (PRC). The identification and differentiation of HSV-1, HSV-2, and VZV occurs by the use of genotype-specific hydrolysis probes. The Lyra assay was performed on the QuantStudio Dx instrument (Life Technologies, Foster City, CA), according to the manufacturer's investigational use only kit's instructions (Quidel Corp., San Diego, CA). For sample preparation, 100 μ l of M4RT medium specimen was placed in a 60°C heat block for 5 min. The heated specimen was mixed with 25 μ l process buffer, and 5 μ l of the mixture was transferred to a microtiter plate well, to which 15 μ l of master mix was added. The plate was then sealed and placed into the QuantStudio Dx instrument for amplification and detection using primers and TaqMan-based probes specific for HSV-1, HSV-2, and VZV.

Laboratory-developed tests. Discordant samples between culture and the Lyra assay were processed and amplified by the Ceph-

eid HSV analyte-specific reagent (ASR) (Sunnyvale, CA) for HSV detection and typing (11) or by the Argene VZV R-gene ASR (bio-Mérieux, Durham, NC) for VZV detection (12). For HSV, 5 μ l of heated specimen was added to 20 μ l of the Cepheid HSV master mix. For VZV, 10 μ l of the heated specimen was added to 15 μ l of the VZV master mix. The tubes were closed and amplified on the SmartCycler II instrument (Cepheid). All positive amplicons were subsequently cloned and confirmed by sequencing (13).

A total of 695 specimens, of which 57 (8.2%) were genital/anal specimens, were included in the study. They were collected from 340 males (48.9%) and 355 females (51.1%), with an average $\pm$ standard deviation age of 52.8 ± 19.6 years. The tube and/or shell vial culture method detected 99 (14.2%) HSV-1/2-positive and 76 (10.9%) VZV-positive swabs. The Lyra Direct assay detected 117 (16.9%) HSV-1/2 and 91 (13.1%) VZV specimens and produced one invalid result, for an invalid result rate of 0.14%. Among the 93 specimens subjected to randomized testing three times on different days, 92 specimens had concordant results, with an overall agreement of 98.9%. One specimen that tested negative in the first two runs was positive for VZV at the third run. The intra-assay % coefficient of variation (%CV) values for the Lyra Direct assay ranged from 0.50 to 0.90% for HSV-1, 0.41 to 1.30% for HSV-2, and 0.29 to 0.75% for VZV (Table 1). The interassay %CV values of the Lyra assay were 2.33% for HSV-1, 2.36% for HSV-2, and 2.61% for VZV (Table 1). In comparison to cell culture, the sensitivity, specificity, and positive and negative predictive values of the Lyra Direct HSV 1+2/VZV assay were 95.0%, 96.1%, 80.3%, and 99.1%, respectively, for HSV-1/2 and 93.4%, 96.8%, 78.0%, and 99.2%, respectively, for VZV (Table 2).

There were 53 specimens with discordant results between culture and the Lyra assay. They were tested with two independent real-time PCR LDT. Among them, 10 specimens were culture positive (5 HSV and 5 VZV)/Lyra assay negative, and 43 specimens were culture negative/Lyra assay positive (23 HSV and 20 VZV). Among the 10 culture-positive/Lyra negative specimens, 9 specimens were negative by both LDT PCR assays (4 HSV and 5 VZV). For the 43 Lyra-positive/culture-negative samples, only one of 20 VZV-positive specimens by the Lyra assay was positive by the LDT-PCR. In contrast, among the 23 specimens that were HSV positive by Lyra assay, 19 (82.6%) were positive by the LDT-PCR. In comparison to a combined standard based on the majority results of the viral culture, Lyra, and LDT-PCR assays, the resolved

TABLE 2 Sensitivity, specificity, PPV, and NPV of the Lyra Direct HSV-1+2/VZV assay ($n = 694$)^a

Viruses by resolution analysis time	No. of specimens by result ^b				Test performance (95% CI) (%) ^c			
	R+ L+	R- L+	R+ L-	R- L-	Sensitivity	Specificity	PPV	NPV
Pre-resolution analysis								
HSV-1/2	94 ^d	23	5	573	95.0 (88.6–98.3)	96.1 (94.2–97.5)	80.3 (72.0–87.1)	99.1 (98.0–99.7)
VZV	71	20	5	598	93.4 (87.8–99.0)	96.8 (95.4–98.2)	78.0 (69.5–86.5)	99.2 (98.4–99.9)
Post-resolution analysis								
HSV-1	68	2	1	623	98.6 (92.3–99.8)	99.7 (98.9–100.0)	97.1 (90.0–99.6)	99.8 (99.1–100.0)
HSV-2	45	2	0	647	100.0 (92.1–100.0)	99.7 (98.9–100.0)	95.7 (85.4–99.4)	100.0 (99.4–100.0)
VZV	72	19	0	603	100.0 (95.0–100.0)	96.9 (95.3–98.2)	79.1 (69.3–86.9)	100.0 (99.4–100.0)

^a PPV, positive predictive value; NPV, negative predictive value.

^b R, reference; L, Lyra. In the pre-resolution analysis, the tube or shell vial culture results were used as references, and in post-resolution analysis, the majority-matched results among culture, Lyra, and LDT were used as references.

^c CI, confidence interval.

^d One specimen was positive for both HSV-1 and HSV-2 by the Lyra assay.

sensitivity, specificity, and positive and negative predictive values of the Lyra assay were 98.6%, 99.7%, 97.1%, and 99.8%, respectively, for HSV-1, 100%, 99.7%, 95.7%, and 100%, respectively, for HSV-2, and 100%, 96.9%, 79.1%, and 100%, respectively, for VZV (Table 2).

The extraction of nucleic acids from specimen is not always necessary to perform before nucleic acid amplification and detection (14). However, this step increases the hands-on time and the turnaround time to results, raises expenses, and potentially introduces contamination. Sakai et al. (14) reported quick detection of HSV-1, HSV-2, and VZV from skin vesicles and exudates without nucleic acid extraction (14). Furthermore, several reports pointed out that omission of the nucleic acid extraction step prior to real-time PCR resulted in a more rapid and cost-effective assay, with little impact upon the sensitivity of detection (9, 14–16). The Lyra device does not require the time-consuming nucleic acid extraction step and simply treats diluted clinical specimens in a 60°C heat block for 5 min. The equipment needed for the Lyra assay reaction is a heat block that furnishes a constant temperature of 60°C and a molecular real-time PCR platform, like the QuantStudio Dx instrument. The Lyra Direct assay consists of simple dilution-only sample preparation and automatic amplification that can bring accurate and rapid HSV-1/2 and VZV diagnostics to a laboratory that lacks cell culture expertise.

Among the 53 specimens with discordant results between culture and the Lyra assay, the Lyra assay detected more HSV (23 samples) and VZV (20 samples) than did culture (5 HSV and 5 VZV samples). When an LDT PCR was used to resolve the discordant results, among the 43 Lyra-positive/culture-negative specimens, 19 Lyra VZV-positive specimens were negative by the LDT-PCR, and only one of the 20 Lyra VZV-positive specimens was positive by the LDT-PCR. In contrast, among the 23 Lyra HSV-positive specimens, 82.6% were positive by the LDT-PCR (8 HSV-1 and 11 HSV-2). Similar to other molecular assays, our data indicate that the Lyra assay is more sensitive than culture for detecting HSV and VZV in lesion specimens from immunocompromised cancer patients. Previous studies have indicated that molecular methods have a significantly higher sensitivity for VZV detection in a variety of clinical specimen types than that of culture (6, 9, 17). After resolution analysis, the Lyra assay had 19 VZV-positive results (2.7%) that were not confirmed by either culture or LDT PCR. Whether these additional VZV-positive findings are related to carryover contamination that occurred during operation merits further investigation (6).

The Lyra Direct HSV 1+2/VZV assay is precise, reproducible, and sensitive for simultaneously detecting and differentiating HSV-1, HSV-2, and VZV in cutaneous and mucocutaneous lesions. Requiring no time-consuming nucleic acid extraction step, the Lyra assay is able to cover three viruses in a single tube with a total sample-to-result time of <60 min.

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