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Multicenter Evaluation of the Simplexa VZV Direct Assay for Detection of Varicella-Zoster Virus in Cerebrospinal Fluid and Lesion-Swab Specimens

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ABSTRACT Varicella-zoster virus (VZV) is the etiologic agent of varicella (chickenpox) and herpes zoster (shingles) infections commonly involving skin, mucous membranes, and less frequently the central nervous system. Traditional methods for the laboratory diagnosis of these infections are time-consuming, labor-intensive, and often insensitive. As such, these tests are being replaced by more sensitive and rapid molecular methods. This study evaluated the performance of two different molecular assays, the Simplexa VZV Direct and Simplexa VZV Swab Direct, to detect VZV DNA in cerebrospinal fluid (CSF) and lesion-swab specimens, respectively. The Simplexa VZV Direct and Simplexa VZV Swab Direct assays were compared against individual composite reference methods that varied depending on the sample cohort examined. A total of 883 CSF and 452 cutaneous and mucocutaneous prospective, retrospective, and contrived specimens were evaluated in this multicenter study. The results of this study showed that the Simplexa assays demonstrated near perfect agreement (k = 0.98) compared to the composite reference methods for the detection of VZV in CSF and lesion swab specimens. A further comparison between the standard of care molecular assays employed at the site of specimen collection and the Simplexa assays demonstrated excellent agreement (k = 1.0). The Simplexa assays offer rapid and reliable alternatives for the detection of VZV in certain clinical specimens without the need for nucleic acid extraction.

KEYWORDS varicella-zoster virus, shingles, swab, cerebrospinal fluid, real-time PCR

Varicella-zoster virus (VZV), formally known as *Human alphaherpesvirus 3*, is the causative agent of varicella (chickenpox) and herpes zoster (shingles). Prior to the introduction of varicella vaccines, VZV caused approximately 4 million infections, primarily among children, each year in the United States, resulting in over 10,000 hospitalizations and up to 150 deaths

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(1). Despite the availability of effective vaccines in many countries, eradication of VZV has yet to be accomplished. Drivers of continued VZV transmission include the import of varicella cases from countries that do not routinely vaccinate, along with vaccine hesitancy and antivaccination sentiment in communities with vaccines available to them. Another contributing factor is reactivation of latent VZV among individuals who either had natural infection or, to a lesser extent, received live-attenuated VZV vaccines earlier in life (2).

VZV is highly contagious and is transmitted by either exposure to infectious respiratory secretions or direct contact with skin lesions. Primary infection results in varicella, which is characterized by fever, malaise, and the appearance of vesicular lesions on the face, trunk, and extremities that usually last for approximately 1 week (1, 3). Following primary infection, VZV establishes a lifelong latent infection of dorsal root ganglia and cranial nerves that can reactivate later in life due to many factors. Reactivation most often manifests as a painful, unilateral vesicular eruption, which typically occurs in a restricted dermatomal distribution. However, the virus can reactivate in multiple dermatomes simultaneously, resulting in widespread distribution (4). Common complications of VZV infections include secondary bacterial infections of the skin and soft tissues. Severe complications, although uncommon, include meningitis, encephalitis, pneumonia (either VZV pneumonia or secondary bacterial pneumonia), bronchitis (either VZV bronchitis or secondary bacterial bronchitis), and visceral dissemination (VZV infection of internal organs including ocular infection), and postherpetic neuralgia may occur and can be life-threatening (3). VZV can also complicate pregnancy; newborns of infected mothers have a small risk of developing congenital varicella syndrome or neonatal varicella (5).

The diagnosis of VZV infections is usually made clinically by observation of the characteristic dermatologic manifestations of varicella and herpes zoster. However, clinical laboratory testing may be indicated to help distinguish VZV infection from Herpes simplex virus and other infections associated with a blistering rash. In patients at risk for severe disease such as encephalitis, laboratory testing is essential and should be used to guide patient management. Likewise, VZV CNS infections can follow either primary infection or reactivation in the absence of zoster rash or detectable lesions on a brain MRI, making it difficult to predict CNS complications (5, 6). The frequency of VZV encephalitis may be underestimated compared to other causes in immunocompromised patients (5). In these settings, rapid diagnosis is essential to provide appropriate patient therapy and support public health measures. Identification of a viral etiology has also been shown to be beneficial, facilitating reduced administration of antibiotics and decreased length of stay in the hospital (2).

The most serious complications of VZV infection arise when the virus reactivates, invading the spinal cord or cerebral arteries, causing myelitis and focal vasculopathies or infection of intra- and extracranial arteries, which may go undetected due to absence of a rash or a CSF pleocytosis (7, 8). Segmental weakness and myelopathy occurring after an episode of herpes zoster is also well recognized (9). The International Herpes Management Forum (IHMF) developed guidelines to aid in the diagnosis and management of CNS syndromes associated with VZV, and recommend that where VZV vasculopathy is suspected, CSF should be evaluated for the presence of VZV using PCR and should also be analyzed for VZV-specific antibodies (10). Several case reports of VZV meningitis confirmed by PCR have been reported, and those cases failed to show any skin manifestation (11). Additionally, in one study, VZV was the most common herpesvirus detected in CSF samples from patients with CNS symptoms, and CSF viral loads were higher in patients with encephalitis and acute aseptic meningitis than in other CNS syndromes caused by VZV (12). In these cases, detection by PCR of VZV DNA and/or anti-VZV IgG antibody in the CSF are instrumental for early therapeutic interventions (13). The ability to quickly identify the pathogen responsible for these serious complications is critical for patient management, as aggressive antiviral treatment can be effective.

Current methods of laboratory testing for VZV include viral culture, serology, direct fluorescent antibody (DFA) testing, and nucleic acid amplification-based assays (6, 14). While viral culture is specific and can help distinguish VZV from other viral pathogens, it takes longer to get results and is much less sensitive compared to the other testing methodologies. Furthermore, many clinical laboratories have moved away from using

viral culture and no longer have the capabilities to perform this test method. Serological tests require paired acute and convalescent specimens, delaying the detection of acute infections. DFA is performed on scrapings taken from the base of lesions and is a rapid and reliable method for diagnosing VZV disease, but this method is not available in all clinical laboratories. Nucleic acid amplification testing (NAAT), the most sensitive test method for VZV, can be used for detecting both invasive disease and local lesions, and is able to detect VZV in a variety of specimen types, including cerebrospinal fluid (CSF) and lesion swabs. However, given the limited number of available FDA cleared assays, PCR testing for VZV is not available in all settings. Most currently used laboratory VZV NAAT methods require nucleic acid extraction, which requires additional instrumentation, processing steps, and technician time. In this multicenter study, we describe an evaluation of new direct PCR-based sample to answer assays designed to detect VZV DNA in CSF and lesion-swab specimens.

MATERIALS AND METHODS

Study design. Fifteen geographically distinct sites across the US participated in this study, and a breakdown of the role of each study participant in onsite testing and specimen collection is outlined in Table S1 in the supplemental material. This clinical study encompassed two main study groups to investigate the performance of the Simplexa VZV Direct assay in CSF specimens and Simplexa VZV Swab Direct assay in cutaneous and mucocutaneous lesion swab specimens. Each study group included an evaluation of several specimen type subgroups, including fresh prospectively collected samples, retrospective banked samples, and contrived samples to supplement low-proportion positive specimens. The transport media types used for the swab study group included UTM (BD UVT), Copan ESwab (Liquid Amies), Remel M4 (ThermoFisher), Remel M4RT (ThermoFisher), Remel M5 (ThermoFisher), and Remel M6 (ThermoFisher).

Each site obtained approval or waiver for local IRB approval based on their role in the study, and all residual samples were de-identified prior to enrollment in the study and testing. Specimens were enrolled, aliquoted, and tested prior to shipping the aliquot to DiaSorin Molecular LLC (Cypress, CA) for comparator testing, and prospective swab specimen aliquots were also sent to the IU School of Medicine for DSFA/DFA testing. Specimen aliquots were stored at refrigerated temperature for up to 7 days after collection (stored at -70^{3} °C or below thereafter) prior to investigational testing. Sample aliquots for sequencing were frozen at −70°C or below and shipped to DiaSorin Molecular LLC on dry ice. DSFA/culture with DFA aliquots were shipped within 1 day of collection at 2–8°C.

Prospective clinical specimens. (i) CSF. A total of 724 prospectively collected CSF specimens were collected over a 9-month period (February–November 2018). Specimens were tested either fresh or after frozen (−70°C) storage using the standard-of-care (SOC) test at each individual site. Residual specimens meeting inclusion criteria, which included submission for routine clinical VZV testing from patients exhibiting signs and symptoms of VZV infection, were selected for the study. Samples that did not meet storage conditions (2−8°C for up to 7 days post collection or at ≤−70°C thereafter) or volume requirements (minimum 300 μL), or had undergone two or more freeze/thaw cycles, were excluded. Six hundred thirty-seven (88%) specimens were included and 87 (12%) specimens were excluded from the study.

(ii) Swabs. A total of 500 prospectively collected lesion swabs were enrolled over a 6-month period (November 2018 – May 2019) from patients with signs and symptoms of cutaneous or mucocutaneous VZV infection. Inclusion criteria included collection and transport of specimens from cutaneous and mucocutaneous lesions in acceptable transport media claimed in the instructions for use (14). Samples were excluded if they did not meet the storage (2–8°C for up to 7 days post collection or at ≤−70°C thereafter) or volume (minimum 1.5 mL) requirements, were collected using calcium alginate swabs, or had undergone two or more freeze/thaw cycles. Inclusion criteria included a requirement for lesions, but a subset of the samples did not have enough details regarding the specific anatomical site to make a cutaneous or mucocutaneous determination, and so they are labeled as unknown in the data sets. Four hundred fifty-two (90.4%) specimens were included and 48 (9.6%) specimens were excluded from the study.

Retrospective clinical specimens. Frozen, banked samples were chosen based on a positive target result using site-specific SOC tests.

(i) CSF. Three CSF preselected specimens positive using the SOC method were included in the retrospective clinical study. In addition, three negative CSF pools were created from banked negative specimens to have sufficient volume. The specimens were blinded, randomized, and distributed to three external laboratories for Simplexa VZV Direct testing.

(ii) Swabs. There were 180 retrospective cutaneous and mucocutaneous lesion swabs collected in UTM (BD UVT) for the clinical study, with 60 (33%) positive specimens and 120 (67%) negative specimens. DiaSorin Molecular LLC performed the Simplexa VZV Swab Direct assay and comparator testing on these specimens.

Contrived specimens. (i) CSF. Contrived specimens were used to supplement the study due to the low proportion of VZV positive CSF clinical samples. A total of 240 CSF contrived specimens were used in this study, including 120 positive specimens contrived by spiking individual negative CSF specimens with VZV Ellen (ATCC# VR-1367) and VZV 9939 (Zeptometrix catalog# 0810170CF) strains (60 specimens per strain) at various concentrations (36 at 2× limit of detection [LoD], 15 at 4× LoD, 3 at 10× LoD, 3 at 20× LoD, and 3 at 50× LoD).

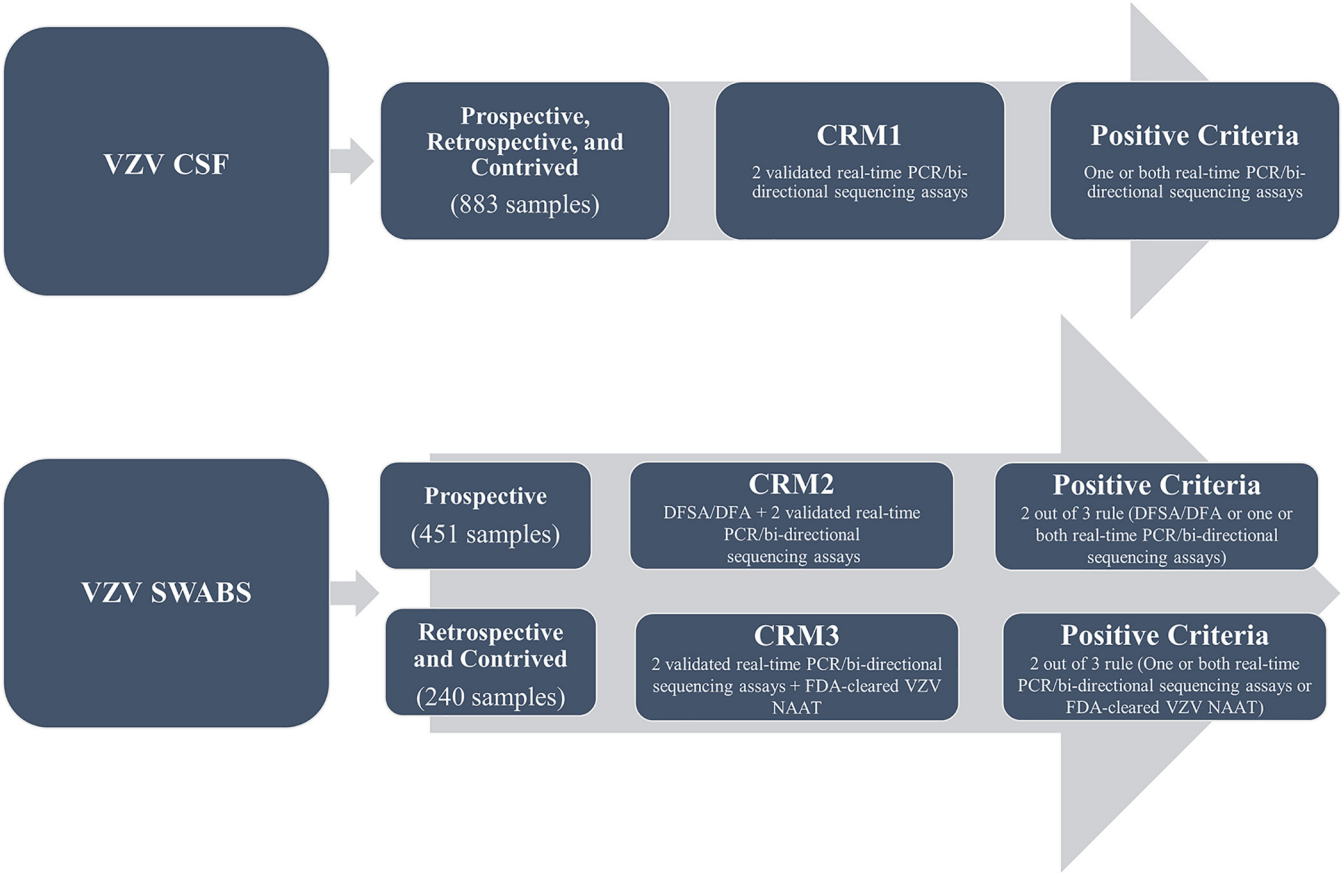

FIG 1 Composite Reference Method (CRM) flowchart.

Also included were 120 pooled negative CSF patient specimens. All contrived samples were blinded, randomized to reduce bias, and sent to four external laboratories for Simplexa VZV Direct testing.

(ii) Swab. Contrived specimens were used to supplement mucocutaneous swab samples due to the low proportion of specimens during prospective collection. There were a total of 120 contrived samples used in this study, including 60 (50%) positive specimens contrived by spiking de-identified negative mucocutaneous UTM (BD UVT) matrix with the VZV Ellen and VZV 9939 strains (30 specimens per strain) at the following concentrations: 18 at 2× LoD, 5 at 4× LoD, 3 at 10× LoD, 2 at 20× LoD, and 2 at 50× LoD. In addition, 60 negative mucocutaneous UTM (BD UVT) matrix specimens were included. All contrived samples were blinded, randomized to reduce bias, and sent to four external laboratories for Simplexa VZV Direct testing.

(iii) Simplexa VZV direct assays. The Simplexa VZV Direct (CSF) and Simplexa VZV Swab Direct assays (DiaSorin Molecular, LLC) were performed on the LIAISON MDX system according to the manufacturer's instructions (15, 16). Briefly, the real-time PCR Simplexa VZV Direct assays enable the direct amplification and detection of VZV DNA from unprocessed cutaneous and mucocutaneous lesion swab specimens or CSF specimens without nucleic acid extraction. Fluorescent probes are used together with corresponding forward and reverse primers to amplify VZV and internal control targets. A well-conserved region of the VZV DNA polymerase gene is targeted to identify VZV DNA in the specimen. An internal control is used to detect PCR failure and/or inhibition.

Performance comparator methods. The performance of the Simplexa VZV assays in different specimen cohorts were compared to a defined composite reference method (CRM), depending on the specific testing requirements of the sample set. A flowchart including the number of samples, the sample cohort, the methods involved, and the criteria for positivity are included in Fig. 1. All CSF specimens, including prospective, retrospective, and contrived, were tested against CRM-1. In clinical practice, viral culture has been utilized as a test method for lesion swab samples, and was incorporated into the CRM for Swab specimens in the form of direct specimen immunofluorescent-antigen testing (DSFA) and/or shell vial culture with direct fluorescent antibody (DFA) confirmation. Given that this method requires fresh samples with viable virus, only prospectively collected Swab specimens were able to utilize DSFA/DFA into CRM-2. Finally, retrospective and contrived Swab specimen performance was compared to CRM-3. All CRMs and their interpretation criteria are outlined below.

(i) CSF. Simplexa VZV Direct results were compared to results from CRM-1 that consisted of two validated real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing, referred to as PCR/bi-directional sequencing assays. Samples were categorized as positive if one or both PCR assays detected VZV DNA with confirmation by bi-directional sequencing

of the amplicons. Samples were categorized as negative if both PCR assays were negative. Samples were tested on Simplexa VZV Direct at the collection sites, and the CRM-1 was performed at DiaSorin Molecular LLC.

(ii) Swab. For Prospectively collected lesion swab specimens, a three-part comparator reference method (CRM-2) that consisted of DSFA and/or culture with DFA for confirmation of VZV isolation and two validated PCR assays followed by bi-directional sequencing was used against the Simplexa VZV Swab Direct investigational assay results, given the requirement of fresh specimens for culture. An alternate three-part CRM (CRM-3) was utilized for both Retrospective and Contrived swab specimens, consisting of two validated PCR assays followed by bi-directional sequencing and an alternative FDA cleared VZV NAAT (Quidel Solana HSV 1 + 2/VZV). The IU School of Medicine performed DSFA and/or culture, and DiaSorin Molecular LLC conducted VZV PCR assay testing followed by bi-directional sequencing. A positive result was determined by applying a minimum two-out-of-three rule for both three-part CRMs outlined here (CRM-2 and CRM-3).

(iii) PCR/Bi-directional sequencing assays. Two independent assays consisting of real-time PCR followed by bi-directional sequencing were developed and validated by DiaSorin Molecular as comparator methods for both the Simplexa VZV Direct (CSF) and Simplexa VZV Swab Direct assays. Each comparator assay targets a discrete region of the VZV DNA polymerase gene and does not overlap with the Simplexa VZV Direct (CSF) or Simplexa VZV Swab Direct assay targets. Each of the four comparator assays were performed by first extracting the sample, either CSF or swab specimen, using a MagNa Pure (Roche), followed by amplification using SYBR green (Qiagen) real-time PCR. The sample was considered VZV positive if the PCR amplicon was generated with a defined melting temperature. The PCR amplicon was bi-directionally sequenced with the same SYBR green PCR primers using BigDye Cycle Sequencing chemistry (Life Technologies). DNA alignment of the bi-directional sequencing results were compared to the GenBank database using a BLAST search to determine whether the sample was positive for VZV. A separate PCR for an internal control was set up to monitor extraction and PCR inhibition, and to validate negative VZV results.

(iv) DFA/culture. Direct immunofluorescence and shell vial culture (DSFA and/or culture isolation with DFA) were used to detect VZV in swab eluates provided in viral transport medium (VTM). Specimens that contained at least 1.2 mL of VTM and that were stored at 2–8°C for less than 72 h from the time of collection were accepted for testing. For direct immunofluorescence, a portion of the VTM was centrifuged for 10 min at 1,600 *g*, and cell pellets were resuspended in 100 μL of supernatant and spotted onto slides. Air-dried smears were fixed in cold methanol and acetone (1:1) prior to staining with the Light Diagnostics Varicella Zoster Virus DFA Kit according to the manufacturer's instructions (EMD Millipore Corp., Temecula, CA). For shell vial culture, 0.3-mL aliquots of well-mixed specimens were inoculated into each of two Super E-Mix ReadyCells (Quidel/Diagnostic Hybrids, Athens, OH) vials. Inoculated vials were centrifuged for 60 min at 700 *g* at room temperature and then incubated for up to 5 days at 35–37°C. One vial from each pair was stained after 2 days of incubation using the Light Diagnostics Varicella Zoster Kit; if VZV was not detected, the remaining shell vial was incubated for an additional 3 days before immunostaining. If VZV was not detected after 5 days of incubation, specimens were considered to be negative.

(v) Limit of detection. Viral stocks of VZV Ellen and VZV 9939 were analyzed to determine copies/mL using a real-time PCR assay calibrated by a copy number standard that was quantitated using Droplet Digital PCR (ddPCR). The LoD was defined as the concentration of virus in copies/mL that resulted in $\geq$95% detection for each strain. The LoD was determined by both percent detection and Probit analysis.

(vi) CSF. A total of eight dilutions of VZV Ellen or VZV 9939 strains in VZV-negative human CSF were examined to determine the LoD for each strain, and 32 replicates of each concentration were tested. The following concentrations were tested: 94 cp/mL, 188 cp/mL, 376 cp/mL, 753 cp/mL, 1,505 cp/mL, 3,010 cp/mL, 6,020 cp/mL, and 12,040 cp/mL for VZV Ellen and 202 cp/mL, 404 cp/mL, 807 cp/mL, 1,614 cp/mL, 3,228 cp/mL, 6,456 cp/mL, 12,912 cp/mL, and 25,824 cp/mL for VZV 9939.

(vii) Swab. A total of five concentrations of VZV Ellen and six concentrations of VZV 9939 were evaluated to determine the LoD for each strain, and 48 replicates of each concentration were tested. All samples were prepared by diluting VZV Ellen or VZV 9939 strains into pooled cutaneous and mucocutaneous lesion swab matrix in UTM that was diluted 1:1 with un-inoculated UTM. The following concentrations were tested: 438 cp/mL, 875 cp/mL, 1750 cp/mL, 3,500 cp/mL, and 7,000 cp/mL for VZV Ellen and 100 cp/mL, 200 cp/mL, 400 cp/mL, 800 cp/mL, 1,600 cp/mL, and 3,200 cp/mL for VZV 9939.

Standard of care assays. Initial testing by the SOC assays was performed at each study site, and the resulting interpretation was used to enroll positive and negative VZV specimens into the clinical study. The SOC assays are not part of the CRMs used in the performance comparisons made against the Simplexa VZV Direct and Simplexa VZV Swab Direct assays. Each SOC method is outlined below, to allow for additional clinical performance comparisons.

(i) FilmArray meningitis/encephalitis (ME) panel. A multiplexed PCR assay was used for the qualitative detection of 14 organisms associated with meningitis or encephalitis, including VZV, from CSF specimens (17). The assay was performed according to the manufacturer's instructions.

(ii) Luminex LDT. A qualitative PCR method was utilized by Marshfield Labs using the VZV MultiCode-RTx Rapid PCR method using primers that amplify a 75 bp target of VZV (orf29). One hundred and ninety-five μL of sample (CSF or swab in transport media) spiked with 5 μL of sample processing control was extracted using the NUCLISENS easyMAG instrument (bioMérieux) and eluted in 50 μL. For each amplification reaction, 5 μL of eluate was added to 20 μL reaction mix (1 μL Luminex Multi-Code ASR VZV primers, 5 μL 5X ISOlution, 1 μL control primer 3, 0.5 μL Titanium *Taq*, and 12.5 μL nuclease-free water). Assays were run using the Roche Cobas z480 Thermocylcer with the following cycling parameters: initial denaturation at 95.0°C for 2 min followed by 45 cycles of amplification using 95.0°C for 5 s, 58.0°C for 20 s, and 72.0°C for 30 s followed by melt analysis. The VZV PCR result was interpreted as positive if the Ct value was $<$39.

(iii) Qiagen LDT. A qualitative PCR method was utilized by The Ohio State University Wexner Medical Center Clinical Microbiology Laboratory to detect VZV using a primer pair targeting the 82 bp region of

tegument protein gene (ORF38 or gene 38). Briefly, 200 μL of CSF or lesion swabs in VTM was extracted using the easyMAG extraction system (bioMérieux) with DNA eluted in 50 μL. For each amplification reaction, 11 μL of eluate was added to 15 μL master mix (0.5 μL Qiagen ASR VZV primers, 11.75 μL PCR solution MBA, 2.25 μL Mag-Sol). Assays were run and analyzed using an ABI 7500 Fast Thermocycler (Applied Biosystems) with the following cycling parameters: denaturation at 95.0°C for 10 min followed by 45 cycles of amplification (95.0°C for 15 s, 60.0°C for 31 s, and 72.0°C for 35 s). VZV was considered detected if the Ct value was $\leq$ 38.

(iv) MCW VZV LDT. A qualitative PCR method developed by Harbecke et al. was utilized by Wisconsin Diagnostic Laboratories to detect VZV in CSF and swab specimens (18). Swab specimens were placed into viral transport media (either Remel M4 or Remel M6) and were stored refrigerated until testing occurred, and CSF specimens were used directly after extraction. Briefly, 200 μL of CSF or swab specimens in VTM were extracted using the easyMAG extraction system (bioMérieux) and the nucleic acid eluted in 50 μL. The amplification reaction mix consisted of 5.4 μL of the nucleic acid eluate and 21.6 μL of the master mix (8.1 μL of VZV mix [primers and probes (IDT)], 13.5 μL ThermoFisher EXPRESS qPCR Supermix). Assays were run and analyzed in an ABI 7500 thermocycler using the following cycling parameters: initial denaturation at 94°C for 10 min followed by 45 cycles of amplification using 94°C for 15 s, 59°C for 30 s, and 72°C for 15 s. VZV was defined as positive if the VZV target Ct value was $\leq$45.

(v) Elitech. A qualitative PCR method was utilized by Beaumont Health to detect VZV in CSF and swab specimens using the EliTechVZV Elite MGB reagents. Briefly, 200 μL of sample plus 10 μL of MGB Alert Internal Control were extracted using the bioMérieux NUCLISENS easyMAG instrument, and the nucleic acid was eluted in 50 μL. For each amplification reaction, 5 μL of this eluate was added to 20 μL reaction mix (1.25 μL 20$\times$ Primer-Probe mix consisting of MGB Alert VZV Primers and MGB Alert VZV Probe, 18.75 μL of MGB Alert 2$\times$ Hot Start PCR Master Mix). Assays were run and analyzed using the ABI 7500 thermocycler with the following cycling parameters: activation of UDG at 50°C for 120 s and initial enzyme activation using 93°C for 120 s, followed by 45 cycles of amplification (93°C for 15 s, 56°C for 30 s, and 72.0°C for 30 s), followed by melt analysis. The VZV result was considered positive if the Ct value was less than 38 and the T_m was within range (T_m = 66.06°C $\pm$ 1.47).

(vi) DiaSorin Molecular LDT. A qualitative PCR method used by the UCLA microbiology laboratory to amplify and detect VZV targets the DNA polymerase gene. This assay is used for cutaneous or mucocutaneous swab specimens using reagents from DiaSorin Molecular. Briefly, 200 μL of each specimen was extracted using the easyMAG (bioMérieux). For each amplification reaction, 5 μL of reaction mix (composed of 4 μL of Universal Master Mix, 0.4 μL VZV Primer Pair, 0.4 μL DI water, and 1 μL SEAC Primer Pair) was combined with 5 μl extracted specimen in a Universal Disc. Assays were run on the LIAISION MDX with the following cycling parameters: 120 s at 97°C initial denaturation followed by 40 cycles of amplification (10 s at 97°C and 30 s at 60°C). Samples with Ct value $<$40 were considered positive.

(vii) Quidel Solana HSV 1 + 2/VZV. The assay utilizes isothermal helicase-dependent amplification (HDA) for the qualitative detection and differentiation of herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), and VZV from cutaneous or mucocutaneous swab specimens (19). The assay was performed according to manufacturer's instructions.

Statistical analysis. Positive percent agreement (PPA), negative percent agreement (NPA), probit, and two-sided (upper/lower) 95% confidence interval (CI) were calculated using Microsoft Office Excel 365 MSO software (Microsoft, Redmond, WA). The percent positive agreement was calculated as TP/(TP + FN) $\times$ 100, the percent negative agreement was calculated as TN/(TN + FP) $\times$ 100, where TP is true-positive results, FN is false-negative results, TN is true-negative results, and FP is false-positive results. Probit analyses were used for the copies/mL determination of the analytical sensitivity study. The dose-response 95th percentile (with 95% confidence interval [CI]) model was assessed using the Finney and Stevens calculations (20). Cohen's kappa values (κ) were also calculated as a measure of overall agreement, with values categorized as almost-perfect ($>$0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20) (21, 22).

RESULTS

CSF clinical agreement. A total of 724 prospective CSF specimens were collected, and 637 were included in the final analysis. Given the low proportion of positive VZV CSF clinical specimens, 6 retrospective and 240 contrived samples were also included, bringing the total of tested samples to 883. The clinical agreement of the Simplexa VZV Direct assay with prospective, retrospective, and contrived CSF samples, combined together, is outlined in Table 1. To assess performance, the Simplexa VZV CSF Direct assay was compared to the CRM-1, consisting of two validated PCR assays, with confirmation of positive amplification with bi-directional sequencing. A CRM-1 result was defined as positive when one or both of the two PCR/bi-directional sequencing assays was positive. The Simplexa VZV CSF Direct assay demonstrated an overall positive percent agreement (PPA) of 100% (95% CI, 0.97–1) and an overall negative percent agreement (NPA) of 99.5% (95% CI, 0.98–1) when compared to CRM-1. Based on further statistical analysis, the agreement between Simplexa VZV CSF Direct assay and CRM-1 was near perfect (kappa = 0.98). Four specimens had false-positive (FP) results in the clinical study (two prospective, one retrospective, and one contrived). One of two prospective FPs was positive via testing with the SOC test at the site of collection, but was negative by both CRM-1 PCR/bi-directional sequencing assays. The discrepant

TABLE 1 Results for Simplexa VZV Direct (CSF) and Simplexa VZV Swab Direct compared to composite reference methods

Simplexa VZV Direct (CSF)	Composite reference method 1[a] (CRM-1)		PPA[b] (±95% CI[d,e])	NPA[c] (±95% CI[d,e])	Kappa (κ)[f]
	Positive	Negative			
Positive	133	4[g]	100% (0.97–1)	99.5% (0.9–1)	0.98 (0.96–1)
Negative	0	746			
Simplexa VZV Direct swab prospective specimens	Composite reference method 2[h] (CRM-2)		PPA[b] (±95% CI[d,e])	NPA[c] (±95% CI[d,e])	Kappa (κ)[f]
	Positive	Negative			
Positive	86	3[i]	97.8% (0.92–0.99)	99.2% (0.98–1)	0.97 (0.93–0.99)
Negative	2[j]	360			
Simplexa VZV Direct swab prospective specimens	DFSA/DFA[k]		PPA[b] (±95% CI[d,e])	NPA[c] (±95% CI[d,e])	Kappa (κ)[f]
	Positive	Negative			
Positive	51	49[l]	96.2% (0.87–0.99)	87.4% (0.84–0.90)	0.60 (0.51 −0.70)
Negative	2[m]	339			
Simplexa VZV Direct swab retrospective & contrived specimens	Composite reference method 3[n] (CRM-3)		PPA[b] (±95% CI[d,e])	NPA[c] (±95% CI[d,e])	Kappa (κ)[f]
	Positive	Negative			
Positive	121	1[o]	99.2% (0.96–1)	99.2% (0.95 – 1)	0.98 (0.96–1)
Negative	1[p]	117			

[a]Composite reference method 1 was defined as the result obtained from two validated real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing.
[b]PPA, positive percent agreement.
[c]NPA, negative percent agreement.
[d]±, upper/lower 95%.
[e]CI, confidence interval.
[f]Almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20).
[g]One of the four discordant samples was positive with an alternate nucleic acid amplification test (NAAT) used by the collection site during routine testing, one was positive via Simplexa VZV Direct with a Ct value of 35.7 and positive by the collection site routine testing method, but negative by the comparator methods, and one was positive via Simplexa VZV Direct assay with a Ct value of 40.1.
[h]Composite reference method 2 was defined as the result obtained from VZV direct stain fluorescent antibody (DSFA) and/or culture isolation with direct fluorescent antibody (DFA) and two PCR/Bi-directional sequencing assays.
[i]Two of three discordant samples were negative by the two PCR/Bi-directional sequencing assays, and one was negative by one of the PCR/Bi-directional sequencing assays and DSFA/DFA.
[j]Both discordant samples were positive by the two PCR/Bi-directional sequencing assays.
[k]DSFA and culture isolation with DFA.
[l]Two of the discordant samples were negative by the two PCR/Bi-directional sequencing assays, one was negative by one of the PCR/Bi-directional sequencing assays, and 46 were positive by the two PCR/Bi-directional sequencing assays.
[m]Both discordant samples were positive by the two PCR/Bi-directional sequencing assays.
[n]Composite reference method 3 was defined as the result obtained from two out of three outcomes from the Quidel Solana HSV 1 + 2/VZV assay and two PCR/Bi-directional sequencing assays.
[o]Discordant sample was negative by one of the two PCR/Bi-directional sequencing assays and the Quidel Solana HSV 1 + 2/VZV assay and positive by the other PCR/Bi-directional sequencing assay.
[p]Discordant sample was negative by the Quidel Solana HSV 1 + 2/VZV assay and positive by the two PCR/Bi-directional sequencing assays.

retrospective specimen was positive by the initial site of collection SOC assay, but was ultimately deemed negative by both CRM-1 PCR/bi-directional sequencing assays. Lastly, one negative specimen from the contrived cohort added for randomization and blinding of specimens was FP via Simplexa VZV Direct assay, with a Ct value of 40.1.

Swab specimen clinical agreement. A total of 500 prospective cutaneous and mucocutaneous swab specimens were collected, and 452 specimens were included in this analysis. The clinical agreement of the Simplexa VZV Swab Direct assay using prospectively collected cutaneous and mucocutaneous lesion swabs was compared to CRM-2, consisting of DSFA and culture isolation with culture confirmation by DFA (DSFA/DFA) and two validated PCR assays followed by bi-directional sequencing to confirm positive PCR amplification (Table 1). A positive result for CRM-2 was considered when two out of three methods were positive. For the 452 prospective clinical specimens, Simplexa VZV Swab Direct assay performance demonstrated an overall PPA of 97.8% (95% CI, 0.92 to 0.99) for mucocutaneous, cutaneous, and unknown lesion samples, and an overall NPA of 99.2% (95% CI, 0.98 to 1) when compared against CRM-2. Furthermore, there was almost perfect agreement between the Simplexa VZV Swab Direct assay and CRM-2 (kappa = 0.97). There were three false FP and two false negative results (FN) in the prospective sample cohort. The two FN samples, one mucocutaneous and one

cutaneous, tested negative by the Simplexa VZV Swab Direct assay and DSFA/DFA, but were positive by both CRM-2 PCR/bi-directional sequencing assays. Two of the three FP cutaneous swab specimens tested positive by the Simplexa VZV Swab Direct assay but were negative by both CRM-2 PCR/bi-directional sequencing assays and DSFA/DFA. The third FP cutaneous swab specimen was found positive by the Simplexa VZV Swab Direct assay and one of the CRM-2 PCR/bi-directional sequencing assays, but was ultimately deemed negative by the other PCR/bi-directional sequencing assay and DSFA/DFA.

The clinical agreement of the prospectively collected VZV cutaneous and mucocutaneous swab specimens compared to DSFA/DFA only (as opposed to the CRM-2) are also highlighted in Table 1. The Simplexa VZV Swab Direct assay performance demonstrated an overall PPA of 96.2% (95% CI, 0.87 to 0.99) and NPA of 87.4% (CI 95%, 0.84 to 0.90) when compared only to the culture-based DSFA/DFA method. There was moderate agreement between the Simplexa VZV Swab Direct assay and DSFA/DFA (kappa = 0.60). Overall, 49 discrepant samples were positive by Simplexa VZV Swab Direct assay and negative by DSFA/DFA method. The breakdown of the discrepant samples was as follows: 4 mucocutaneous, 44 cutaneous, and 1 swab of unknown origin. Alternatively, two discrepant samples were positive by the DSFA/DFA method and negative by the Simplexa VZV Swab Direct assay.

The performance of retrospective and contrived cutaneous and mucocutaneous swab specimens are combined and presented in Table 1. They are included separately since the CRMs used to examine the retrospective (CRM-3) and prospective (CRM-2) samples differ due to the requirement for fresh viable VZV samples using the DSFA/DFA method. The alternative CRM-3 was implemented for the previously frozen retrospective and contrived specimens, incorporating an FDA-cleared NAAT (Quidel Solana HSV 1 + 2/VZV) instead of DSFA/DFA, and the same two PCR/Bi-directional sequencing assays utilized in CRM-2. The overall interpretation of CRM-3 was defined as the result obtained from two out of three outcomes. The inclusion of these data supplements the low proportion of positive mucocutaneous specimens collected prospectively. The Simplexa VZV Swab Direct assay performance in combined retrospective and contrived specimens showed an overall PPA of 99.2% (CI 95%, 0.96–1) and an overall NPA of 99.2% (CI 95%, 0.95–1) when compared against CRM-3. There was almost perfect agreement between the Simplexa VZV Swab Direct assay and CRM-3 (kappa = 0.98). There was a total of two discordant specimens, one mucocutaneous and one cutaneous, in the retrospective cohort. The FN mucocutaneous specimen was negative by the Simplexa VZV Swab Direct and Quidel Solana HSV 1 + 2/VZV assays and positive by both CRM-3 PCR/Bi-directional sequencing assays. The FP cutaneous specimen was positive by the Simplexa VZV Swab Direct assay and one of the CRM-3 PCR/Bi-directional sequencing assays but negative by the Quidel Solana HSV 1 + 2/VZV assay and the other PCR/Bi-directional sequencing assay.

Prospective positive sample Ct distribution for CSF and swab specimens. VZV CNS infection is a rare complication of viral reactivation, or less commonly primary infection, although the outcome can be severe. Cutaneous or mucocutaneous lesions are the hallmark symptom of a VZV infection. The distribution of the Simplexa VZV Direct and Simplexa VZV Swab Direct assay cycle threshold (Ct) values from the prospectively collected clinical CSF, cutaneous, and mucocutaneous swab specimens have been included in this study (Fig. 2). Although the assays are qualitative, a general comparison of the viral burden in CNS compartment and skin lesions can be made. There was a 10 Ct difference, based on the mean, between the CSF and cutaneous or mucocutaneous specimens, suggesting the VZV viral load is higher in lesions. The cutaneous and mucocutaneous Ct values had similar mean values and distribution.

LoD of CSF and swab specimens. The LoD was defined as the minimum concentration with detection values of ≥95% by percent positive rate and ≥95% by probit analysis. The CSF LoD established by percent positive rate ranged from 1,505 to 1,614 copies/mL when using the VZV Ellen and 9939 strains, respectively. The LoD results were further subjected to probit analysis. The 95% detection limit values were 928 ± 33 copies/mL for the VZV Ellen strain and 1,181 ± 42 copies/mL for the VZV 9939 strain (Table 2A).

The swab LoD established by percent positive rate ranged from 3,500 to 800 copies/mL when using the VZV Ellen and 9939 strains, respectively. The LoD results based on probit

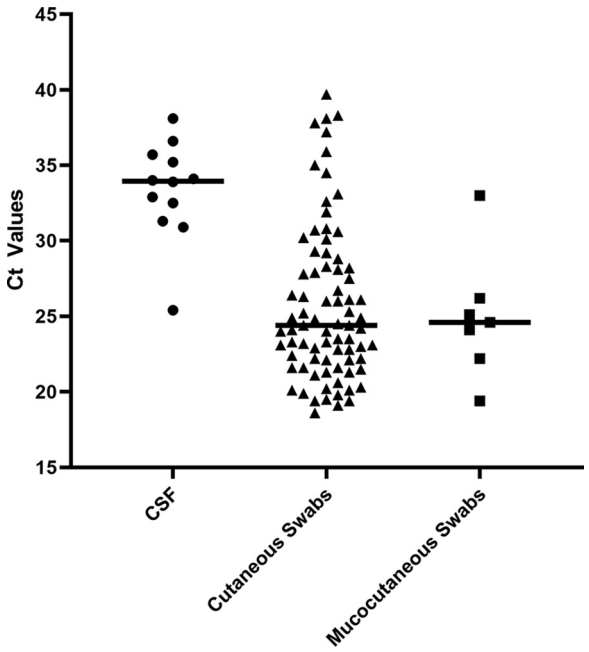

FIG 2 Distribution of Ct values from prospective positive CSF and swab specimens.

analysis were as follows: the 95% LoD value for the VZV Ellen strain was 1,992 ± 93 copies/mL, and 752 ± 86 copies/mL for the VZV 9939 strain (Table 2B).

Alternate molecular methods comparison with Simplexa VZV Assays. (i) Standard-of-care assays. In addition to the performance comparisons made between the Simplexa VZV Direct assays and the previously outlined CRMs, further comparisons with the site-specific SOC methods used to initially characterize the samples were also examined in this study. Given that no other commercially available molecular VZV assay has claims for both CSF and lesion swab specimens, and the testing options for individual specimen types are limited, as evidenced by the large number of LDTs utilized as the SOC method at the clinical testing sites, each CRM utilized internally validated PCR/Bi-directional sequencing assays for initial performance comparisons. However, further performance comparisons between the Simplexa VZV Direct assays and the SOC methods offer additional clinically relevant information.

A comparison of Simplexa VZV Direct assay performance in CSF specimens compared to the SOC assay initially used to characterize the samples at each sample collection site is summarized in Table 3. The clinical comparison includes commercially available NAATs, both

TABLE 2 Limit of detection

	Positive rate %				Probit (±95% CI)[a,b]	
Strain	Copies/mL	Copies/Rx	% Detection	Mean Ct ± SD (%CV)	Copies/mL	Copies/Rx
Summary of limit of detection results for Simplexa VZV Direct (CSF)						
Ellen	1,505	15	96.8% (31/32)	37.8 ± 0.9 (2.4%)	928 ± 33	9
9939	1,614	16	100% (32/32)	37.0 ± 1.6 (4.2%)	1,181 ± 42	12
Summary of limit of detection results for Simplexa VZV Swab Direct						
Ellen	3,500	35	100% (48/48)	35.6 ± 0.9 (2.4%)	1,992 ± 93	20
9939	800	8	95.8% (46/48)	38.7 ± 1.2 (3.0%)	752 ± 86	8

[a]CI, confidence interval.
[b]±, upper/lower 95%.

TABLE 3 Clinical performance comparison of standard of care methods in CSF specimens

Molecular assay	Simplexa VZV direct		PPA[a] (± 95% CI)[d,e]	NPA[b]	Kappa (κ)[c]
	Positive	Negative			
BioFire ME Panel					
Positive	7	0	100%	100%	1.00
Negative	0	501	(0.57–1)	(0.99–1)	(1–1)
Luminex LDT					
Positive	2	0	100%	100%	1.00
Negative	0	26	(0.34–1)	(0.88–1)	(1–1)
QIAGEN LDT					
Positive	0	0	N/A[f]	100%	N/A[f]
Negative	0	14		(0.77–1)	

[a]PPA, positive percent agreement.
[b]NPA, negative percent agreement.
[c]Almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20).
[d]±, upper/lower 95%.
[e]CI, confidence interval.
[f]N/A, not applicable.

FDA-cleared (FilmArray) and LDT/RUO (Luminex, Qiagen). The PPA and NPA were both 100% when comparing the performance of Simplexa VZV Direct with the three SOC assays, and the agreement was almost perfect (kappa = 1.0). All three comparator assays use >50 μL sample input and require front-end extraction compared to Simplexa VZV Direct that uses only 50 μL CSF with no separate extraction step.

A performance comparison of the Simplexa VZV Swab Direct assay in swab specimens to the SOC assay initially used to characterize the samples at each sample collection site is outlined in Table 4. Clinical performance compared to the available real-time PCR or isothermal DNA amplification methods commercially available, both FDA-cleared (Quidel Solana, Luminex ARIES) and LDT/RUO (DiaSorin Molecular LDT, Qiagen, MCW LDT, ELITech). Both the PPA and NPA were 100%, and there was nearly perfect agreement (kappa = 1.0). All

TABLE 4 Clinical performance comparison of standard of care methods in cutaneous and mucocutaneous swab specimens

Technology/assay	Simplexa VZV Direct		PPA[a] (± 95% CI)[d,e]	NPA[b]	Kappa (κ)[c]
	Positive	Negative			
Helicase dependent amplification					
Quidel Solana HSV 1 + 2/VZV					
Positive	3	0	100%	100%	1.00
Negative	0	133	(0.44–1)	(0.97–1)	(1–1)
Real-time PCR amplification					
Luminex Aries					
Positive	15	0	100%	100%	1.00
Negative	0	34	(0.78–1)	(0.90–1)	(1–1)
DiaSorin Molecular LDT					
Positive	19	0	100%	100%	1.00
Negative	0	20	(0.83–1)	(0.84–1)	(1–1)
QIAGEN LDT					
Positive	9	0	100%	100%	1.00
Negative	0	14	(0.70–1)	(0.78–1)	(1–1)
MCW LDT					
Positive	14	0	100%	100%	1.00
Negative	0	29	(0.79–1)	(0.88–1)	(1–1)
ELITech VZV ELITe MGB Kit					
Positive	29	0	100%	100%	1.00
Negative	0	38	(0.88–1)	(0.91–1)	(1–1)

[a]PPA, positive percent agreement.
[b]NPA, negative percent agreement.
[c]Almost-perfect (>0.90), strong (0.80 to 0.90), moderate (0.60 to 0.79), weak (0.40 to 0.59), minimal (0.21 to 0.39), or none (0 to 0.20).
[d]±, upper/lower 95%.
[e]CI, confidence interval.

comparator assays use >50 μL sample input, and all methods require front-end extraction, except Solana. which requires a separate sample preparation by heating before amplification, compared to Simplexa VZV Swab Direct, which uses 50 μL swab specimen with no separate extraction, preparation, or heating step.

DISCUSSION

Herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), and varicella-zoster virus (VZV) are difficult to distinguish due to their common clinical presentations (14, 23, 24). A misdiagnosis, particularly when misreporting as either noninfectious or bacterial etiology, not only puts an immunocompromised patient in jeopardy of developing life-threatening complications, but it delays the administration of appropriate antiviral treatment and increases the likelihood of nosocomial transmission (25, 26). Therefore, the development of rapid and sensitive tests, such as the Simplexa VZV Direct and Simplexa VZV Swab Direct assays, are critical to both therapeutic decision-making and infection control measures.

Inclusion of prospective, retrospective, and contrived CSF and swab specimens allowed for a larger and more diverse specimen pool than that of previous studies. Each specimen type fell within 100% PPA and 99.5% NPA with its comparator methods (Table 1). The study also included the demographic data on prospective and retrospective specimens, establishing the Simplexa VZV assays performance characteristics of 100% PPA and 100% NPA versus routine comparator methods, including male and female specimens from age groups ranging from newborn to greater than 60 years of age (data not shown).

The Simplexa VZV Swab Direct assay upholds the same level of sensitivity as DSFA/DFA but does not impose the difficultly or constraint to obtain a viable sample. DSFA/DFA requires intact, infected cells and can often produce inconclusive results if the sample was not obtained or transported properly. In our study, when the culture-based DSFA/DFA method was compared to the Simplexa VZV Swab Direct assay, a high number of discrepant samples that were positive by the direct molecular assay and negative by DSFA/DFA were observed. Various factors can attribute to these discrepant results. Both the viability and viral load of VZV plays a large role in the sensitivity of culture-based detection methods. Many factors can affect the viability of VZV, including storage and transport considerations, and the stage of infection, from early-stage blisters to scabbed lesions, has a large impact on viral load. Notably, PCR-based methods do not have the same stability or viability constraints. The literature has described a shift away from cell culture in clinical VZV testing partly due to the lower sensitivity compared to PCR methods (26, 27). When compared to the CRMs, the Simplexa assays performed exceptionally well using a wide variety of transport media types demonstrating robust performance utilizing direct chemistry without the need for extraction (Table S2). Additional media equivalency conducted across a range of transport media types as well as ESwab showed all swab/transport media combinations performed well, with similar Ct values (data not shown). The inclusion of the range of media types attests to the reliability of different transport media that may be encountered in routine practice or during media shortages that were frequently encountered during the COVID-19 pandemic for performance of the Simplexa VZV Swab Direct testing.

Clinical performance of the Simplexa VZV assays compared to the available real-time PCR or isothermal DNA amplification methods in the field, both FDA-cleared (FilmArray ME Panel, Quidel Solana), and LDT/RUO (DiaSorin Molecular LDT, Qiagen, MCW LDT, ELITech, Luminex) assays demonstrated 100% PPA and NPA (Tables 3 and 4). All methods require front-end extraction except Solana, which requires a separate sample preparation step by heating before amplification, and Simplexa VZV Swab Direct, which does not require a separate extraction or heating step. Direct PCR methods have several advantages, including reducing the time and cost for processing specimens, increasing testing throughput, and decreasing the risk of cross-contamination. Furthermore, all comparator assays used >50 μL sample input compared to the requirement of only 50 μL for both Simplexa assays. Some of the comparator assays can test for both HSV and VZV at the same time, such as the Lyra Direct HSV 1 + 2/VZV assay (Quidel Corporation). Fan et al. evaluated the Lyra Direct HSV 1 + 2/VZV multiplex real-time PCR assay for the detection and differentiation of herpesviruses on 695

consecutive cutaneous and mucocutaneous lesion specimens with sensitivities and specificities of 93.4 to 95.0% and 96.1 to 96.8%, respectively, in comparison to those of viral culture (26). Granato et al. performed a prospective 1-year study on 2,113 cutaneous and mucocutaneous specimens using Lyra Direct HSV 1 + 2/VZV Assay and had an unexpected detection of VZV DNA in 11.1% of overall positives contributed from genital sites (24). Their study suggested that VZV infection of the genital area may not be as uncommon as previously thought, and VZV testing from genital sites may have a considerable impact on patient counseling and public health considerations (24). The Simplexa HSV 1 & 2 Direct assay can be run simultaneously with the Simplexa VZV Swab Direct assay described in this study in order to provide simultaneous HSV and VZV results from genital swabs, but the combination use was not addressed during this study.

The relationship between viral loads (Ct values) from VZV infections in the three different clinical specimen types collected prospectively, CSF and cutaneous and mucocutaneous swabs, is examined in Fig. 2. Based on the data, VZV infections in this study reflect a wide range of viral loads, from high to low within the tested population. In general, given that the Simplexa VZV assays are qualitative, the viral loads associated with the CNS compared to skin lesions are much lower, as seen by the higher average Ct value associated with CSF specimens, which is consistent with clinical expectations. Several studies have shown that the viral loads found in patients with meningitis are lower than encephalitis. Aberle et al. reported that significantly higher viral loads (geometric mean [GM]: 7.2×10^4 copies/mL) were found in patients with encephalitis compared to patients with meningitis (GM: 4.1×10^3 copies/mL) (28). Similarly, another study conducted by Persson et al. demonstrated a higher level of VZV DNA in the encephalitis group and in the acute aseptic meningitis group than in the cranial nerve affection group ($P = 0.015$ and $P = 0.006$, respectively) (12). In comparison, the cutaneous and subcutaneous lesions have much higher levels of VZV DNA. Our studies confirmed these findings where higher Ct values were observed in CSF compared to non-CSF sources. The Simplexa VZV Direct and Simplexa VZV Swab Direct assays underscore the importance of having a suitable and reliable assay for the detection range of these infections. Even though the number of mucocutaneous lesion swab samples obtained prospectively was limited in comparison to cutaneous swabs, the average Ct values, denoted by the black bar (Fig. 1), are strikingly similar, showing that any additional inhibitors from mucocutaneous samples do not adversely affect performance.

Our study has several advantages. To our knowledge, this is the first and largest systematic study for detection of VZV DNA in CSF samples without the need of nucleic acid extraction prior to amplification. Because a vesicular rash is often absent in patients with herpes zoster-associated neurological disease, it is important to consider VZV in the differential diagnosis of patients with unexplained neurological symptoms. Due to availability of PCR, VZV is diagnosed as one of the most common viruses causing CNS disease that includes a wide spectrum of neurological manifestations. Availability of rapid diagnostics for suspected VZV CNS infection like Simplexa VZV Direct may aid in expedited and targeted patient management. This is also the first direct-from-swab study, to our knowledge, that includes a wide spectrum of transport medium types. Additionally, this study is robust, as multiple comparator methods and technologies were used for both Simplexa Direct and Simplexa Swab Direct assays. Lastly, we also assessed the analytical specificity of the Simplexa VZV Direct and Simplexa VZV Swab Direct assays and determined that there was no cross-reactivity with other common or closely related pathogens (15, 16).

One limitation is that the Simplexa VZV assays only detect one herpesvirus. The development of a rapid assay that could detect and differentiate between the three human herpesviruses commonly associated with skin lesions and CNS involvement, namely, HSV-1, HSV-2 and VZV, could significantly help with proper diagnosis and save time from having to perform multiple tests to narrow down the specific virus infecting the host (24). However, DiaSorin Molecular does have a separate assay for the detection of HSV 1 & 2 in both CSF and lesion swab specimens. Additionally, our study was limited in the number of clinical CSF VZV positive specimens collected, although this number was supplemented by inclusion of retrospective and contrived specimens. Another limitation is that Simplexa VZV Direct is a not a quantitative

assay. So far, the association between viral load in the CSF of patients with primary or reactivated VZV but without neurological symptoms is not known. However, in a small study in patients with postherpetic neuralgia (PHN), the persistence of higher viral loads over time in peripheral blood has been associated with a longer time to recovery (29). Quantitative assays may have implications in monitoring response to therapy.

Overall, detection of VZV using Simplexa VZV Direct and Simplexa VZV Swab Direct assays offers reliable and rapid results for the diagnosis of VZV-related diseases without the need for nucleic acid extraction, immunofluorescence, or time-consuming viral culture.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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