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A comparison of the Quidel Solana HSV 1 + 2/VZV Assay, the Focus Diagnostics Simplexa HSV 1 & 2 Direct Assay and the Luminex Aries HSV 1& 2 Assay for detection of herpes simplex virus 1 and 2 from swab specimens

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ABSTRACT

Background: Molecular methods enable more rapid and sensitive detection of herpes simplex virus (HSV) than viral culture.
Objective: Three commercial molecular methods, all of which detect both HSV-1 and HSV-2, were compared to viral culture for the detection of HSV from swab specimens.
Study design: Pediatric and adult patient viral swab specimens were cultured for HSV. Residual swab fluid was frozen at −80 °C until tested with the 3 molecular methods: the Quidel Solana HSV 1 + 2/VZV Assay, the Focus Diagnostics Simplexa HSV 1 & 2 Direct Assay and the Luminex Aries HSV 1&2 Assay. A true positive was defined as positive by culture or positive by ≥ 2/3 molecular methods.
Results: 177 specimens were studied. The sensitivity of culture was 81.3% (61/75, 95% CI 70.7–89.4%) and specificity was 100% (102/102, 95% CI 96.4–100%). The sensitivities of both the Solana and Simplexa were 100% (75/75, 95% CI 95.2–100%) and specificities were also both 100% (102/102, 95% CI 96.4–100%). The Aries had a sensitivity of 98.7% (74/75, 95% CI 92.8–99.97%) and specificity 99.0% (101/102, 95% CI 94.7–99.98%). All three molecular methods were significantly more sensitive than culture ($p \leq 0.0005$ for Solana and Simplexa and $p \leq 0.0012$ for Aries).
Conclusion: All the molecular methods studied provided a significantly higher sensitivity than culture. In addition, the molecular methods took 1–2 hours to perform compared to a mean of 2.1 days for culture results. Use of any of the three molecular methods could lead to improved patient care.

1. Background

Herpes simplex virus (HSV) infections are caused by HSV-1 and 2, two members of the *Herpesviridae* family. HSV infections are a significant global health problem. It is estimated that 19.2 million new HSV-2 infections occurred among adults and adolescents aged 15–49 years worldwide in 2012, and an estimated 417 million people were HSV-2 carriers globally in 2012 [1]. Herpes simplex virus type 2 (HSV-2) was formerly the most common cause of genital ulcers and HSV type 1 (HSV-1) typically caused non-sexually-transmitted oral herpes infections. However, it is increasingly noticed that HSV-1 causes a substantial portion of genital HSV infections, especially in developed countries, and now accounts for at least 50% of genital HSV in USA [2]. Globally, an estimated 140 million people had genital HSV-1 infections in 2012 [1].

Although HSV is often diagnosed clinically, laboratory testing is necessary for cases where the diagnosis is uncertain. Historically, viral culture was considered the reference standard for HSV diagnosis. However, in more modern times, culture has been found to be less sensitive than nucleic acid amplification tests (NAATs) [3]. The use of NAATS also provides faster results than culture, which may be important for patient management and initiation of anti-viral medications. Accurate diagnosis through laboratory testing is especially important in pregnant women, given the risk of severe disease in the neonate [4],

Abbreviations: HSV, herpes simplex virus; VZV, varicella zoster virus; PCR, polymerase chain reaction; HAD, helicase dependent amplification; CI, confidence interval; Ct, cycle threshold; NAAT, nucleic acid amplification test

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and in immunocompromised patients, such as persons with HIV.

There are several commercially available NAATs for HSV. As described below, we undertook an evaluation of three such tests, the Quidel Solana HSV 1 + 2/VZV Assay, which uses an isothermal amplification method known as helicase dependent amplification (HDA) for target detection, and the Focus Diagnostics Simplexa HSV 1 & 2 Direct Assay and Luminex Aries HSV 1&2 Assay, which both use PCR.

Of note, one of the assays being investigated, the Solana HSV 1 + 2/VZV Assay, also detects varicella zoster virus (VZV) in the same reaction as HSV-1 and 2. VZV detection with this assay was therefore compared to VZV detection by viral culture.

2. Methods

2.1. Study location

This study was conducted at the Children's Hospital of Eastern Ontario (CHEO) and The Ottawa Hospital, ON Canada, which serve a catchment area of 1.5 million people. This study was approved by the CHEO REB (Research Ethics Board) CHEO 18/63×. The remainder of specimen swab fluid that would have otherwise been discarded was used for testing, therefore individual patient consent was not required by the REB. Patient age, gender, and specimen site, as recorded on the requisition, were recorded.

2.2. Study specimens

Swab specimens were collected from patients who presented with cutaneous or mucocutaneous lesions using Multitrans™ Collection and Transportation System (Starplex Scientific, Etobicoke, ON) polyester swabs with liquid media. These swabs were set up for viral culture and the remainder of the swab fluid was then frozen at −80 °C until used for molecular methods.

Specimen inclusion and exclusion criteria: All swab specimens that were sent to the Regional Virology Laboratory of CHEO from March to May of 2018 were included in the study. If multiple swabs of the same patient from the same day were collected, only the first swab was included in the study. Other specimen types such as cerebrospinal fluid and bronchial alveolar lavage were not included in the study.

2.3. Culture methods

HSV/VZV viral culture was performed using MRC-5 (Medical Research Council cell strain 5), a diploid human lung fibroblast culture line (ATCC® CCL-171™) [5]. Cells were inoculated with the swab specimen transport medium, and monitored for 7 days for HSV growth and up to 10 days for VZV. If cytopathic effect was observed, fluorescent antibody testing with HSV-1, HSV-2, and VZV specific antibodies was performed using commercial antibodies (Herpes Simplex Virus Identification and Typing Kit, Quidel Diagnostic Hybrids - D3 DFA, Merifluor VZV immunoreagent, Meridian BioScience, Inc.) for confirmation and species identification.

2.4. Molecular methods

All molecular methods were performed according to the manufacturer's guidelines. Briefly, the Solana method, unlike the other two molecular methods, required an initial brief specimen lysis step in which 20 µL of swab fluid was added to a buffer which was then incubated at 95 °C for 5 min. Once the specimen was lysed, it was added to the reaction tubes containing the lyophilized HDA reagents, and run in the instrument. The reaction time was approximately 50 min. Up to 12 specimens could be performed per run with a single instrument. A competitive process control was used to monitor for inhibitory substances, reagent failure and instrument failure.

With the Simplexa method, 50 µL of the specimen was placed in the specimen well of the Direct Amplification Disc and 50 µL of the thawed assay was placed in the reagent well. The reaction was then run, taking approximately 60 min. Up to 8 specimens could be tested per run with a single instrument. An internal control was used to detect PCR failure and/or inhibition.

For the Aries test, 200 µL of the specimen was loaded into one cassette containing all the required reagents for extraction, purification and amplification, as well as a specimen processing control to monitor for inhibition or PCR failure. Up to 12 specimens could be tested per run with one device, and run time was approximately 2 h.

2.5. Laboratory-developed test for VZV

As noted, a VZV laboratory-developed test method was also performed on discordant specimens that tested positive for VZV by the Solana assay but were negative by culture, Briefly, specimen DNA was obtained with an automated nucleic acid extraction device (MagNA Pure Compact, Roche Canada, Laval, QC) and real-time PCR was performed using a published VZV real-time PCR assay [6] on a LightCycler (Roche Canada) thermocycler.

2.6. Statistical analysis

We hypothesized that the molecular methods being investigated would have higher sensitivity than viral culture, based on previous studies conducted [3]. We therefore elected to define a true positive result as one that was positive for HSV-1 or 2 by culture or positive for HSV-1 or 2 by ≥ 2/3 molecular methods. A true negative result was defined as negative for HSV-1 or 2 by culture and by ≥ 2/3 molecular assays.

To verify whether a discrepant VZV Solana-positive VZV culture-negative specimen was a true positive, the discrepant specimens were tested against a laboratory-developed VZV PCR assay. A true positive VZV result was defined as one that was culture positive or if culture negative, was positive by both the Solana and the laboratory-developed VZV PCR assay. Test performance characteristics and 95% confidence intervals (CI) were calculated using MedCalc software [7]. In order to have a larger number of positive samples for analysis, we chose to assess sensitivity and specificity for the combined HSV-1 and HSV-2 results rather than analyze each virus separately. The McNemar test, a statistical method used to compare results for paired specimens, was used to analyze differences between the molecular assays and viral culture methods [8].

3. Results

177 swab specimens were included in the study. Table 1 shows the breakdown of specimen site by age group. In total, 141 (79.7%) swabs were from adults and 36 (20.3%) swabs were from children (defined as < 18 years of age). The mean patient age was 34.7 years. 62.1% of specimens were from females, 37.2% from males, and, for one specimen, patient sex was not recorded. 90/177 (50.8%) specimens were from non-genital sites and 72/177 (40.7%) were from genital sites. For 15/177 (8.5%) specimens, swab site was not indicated.

Table 1
Herpes simplex virus specimen sites by age group.

Specimen site	Age group		
	Pediatric (< 18 years of age)	Adult (≥ 18 years of age)	Total (n = 177)
Genital	10	62	72
Non-genital	22	68	90
Not specified	4	11	15
Total	36	141	177

Table 2
Performance characteristics for HSV detection by viral culture and by molecular assays.

Method	Performance Indicator		
	Sensitivity % (95% CI)	Specificity % (95% CI)	Accuracy % (95% CI)
Viral culture	81.3 (70.7–89.4)	100 (96.4–100)	92.1 (87.1–95.6)
Quidel Solana HSV 1 + 2/VZV	100 (95.2–100)	100 (96.4–100)	100 (97.0–100)
Focus Diagnostics Simplexa HSV 1 & 2 Direct	100 (95.2–100)	100 (96.4–100)	100 (97.0–100)
Luminex Aries HSV 1&2	98.7 (92.8–100)	99 (94.7–100)	98.9 (96–99.9)

Using the HSV consensus definition of positivity, there were 75/177 (42%) true positive specimens for HSV-1 or 2 (62/75 (83%) HSV-1, 13/75 (17%) HSV-2). Performance characteristics for the three molecular assays and viral culture are shown in Table 2. Of note, the sensitivity of culture was considerably lower than that of the molecular assays at 81.3% (61/75, 95% CI 70.7–89.4%). The mean time to a positive viral culture was 2.1 days.

The performance of the Solana and Simplexa assays was identical, both giving sensitivities of 100% (75/75, 95% CI 95.2–100%). Specificities were also both 100% (102/102, 95% CI 96.4–100%). The Aries assay also had high sensitivity and specificity, but gave one false positive and one false negative result. Aries therefore had a sensitivity of 98.7% (74/75, 95% CI 92.8–100%) and specificity was 99.0% (101/102, 95% CI 94.7–100%).

Using the study definition of true positives, the results of culture were compared to each molecular test and analyzed with the McNemar test (8). All three molecular tests were significantly more sensitive than culture, with $p \leq 0.0005$ for the Simplex and Solana assays and $p \leq 0.0012$ for the Aries assay.

With respect to VZV, which was solely detected by the Solana test, only 1 specimen grew VZV in culture, while 12 specimens were positive by the Solana VZV assay. Eleven of these 12 were also positive with the laboratory-developed VZV assay, and were therefore considered as true positives. The sensitivity of the Solana in its detection of VZV was therefore 100% (95% CI 71.5–100%) and specificity was 99.4% (95% CI 96.7–99.9%). The accuracy of the Solana for VZV detection was 99.4% (95% CI 96.9–99.9%). According to the McNemar test, the Solana VZV assay was significantly more sensitive than culture ($p \leq 0.0026$). Of interest, one of the 11 (9%) of the VZV positive specimens confirmed as a true positive was collected from a genital site, similar to the proportion of genital VZV reported in a previously published study [9].

Of note, no results were called as invalid by the Solana assay. Both the Simplexa and the Aries reported one specimen as invalid (these were different specimens for each test). The invalid specimens were repeated once and both specimens then gave valid results.

4. Discussion

Our findings agree with previous reports demonstrating that molecular methods have a higher sensitivity than viral culture for HSV-1 and 2 [3]. All three commercial assays showed both very high sensitivity and specificity for HSV detection. In terms of discordant results, the Aries assay had one false positive result. This was a positive result for HSV-1 in a cutaneous specimen from a pediatric patient which was negative for HSV by culture and with the other two molecular assays. Of note, this same specimen was positive for VZV by the Solana assay and the laboratory-developed VZV assay. The Aries also had one false negative result. This was from a genital specimen that was positive for HSV-1 by viral culture and by the other two molecular assays.

With respect to the literature specific assays used in this study, to our knowledge there are no published papers on the performance of the Solana HSV 1 & 2/VZV assay. As described above, we found this assay to have a high sensitivity and specificity for the detection of both HSV and VZV. Among the molecular systems being investigated, the technology of the Solana is unique in that it utilizes (isothermal) helicase dependent amplification (HDA) while both the Aries and the Simplexa assay use the polymerase chain reaction (PCR). In addition, the Solana required a brief nucleic acid (NA) extraction step prior to the amplification reaction, while the Simplexa and Aries tests are load and go assays performed directly on clinical specimens.

With respect to the Simplexa HSV 1 & 2 assay used in this paper, one earlier study of swab specimens has been reported. Their results demonstrated a sensitivity of 94.8% for the detection of HSV-1 or 2, and a specificity of 100% [10]. In a second study, separate Focus Simplexa analyte specific reagent assays for HSV-1 and 2 and VZV were combined into a single multiplex reaction, and swab and non-swab specimens such as cerebrospinal fluid (CSF) and bronchoalveolar lavage (BAL) fluids were studied. The sensitivities for HSV-1, HSV-2 and VZV were each reported as 100% with specificities of 96.8% for HSV-1 and 100% for HSV-2 and VZV [11]. Of note, although not assessed in our study, the performance of the Simplexa for detection of HSV in CSF specimens has also been evaluated in other studies [12,13] and the assay is FDA cleared for CSF testing unlike the Aries and the Solana assays.

For the Aries assay, a multicenter evaluation reported a sensitivity of 91.1% for HSV-1 and 95% for HSV-2 for cutaneous lesions and 97% for HSV-1 and 98.5% for HSV-2 mucocutaneous lesions with specificities of 94.2% for HSV-1 and 88.8% for HSV-2 (cutaneous) and 95.4% for HSV-1 and 93.2% for HSV-2 (mucocutaneous) compared to the ELVIS HSV ID and D3 Typing Test System [14]. In as second study comparing three commercially-available platforms, the sensitivity of the Aries was reported as 100% for both HSV-1 and HSV-2 with specificities of 99.5% and 99.5% for HSV-1 and HSV-2 respectively [15].

Each method has its own advantages and disadvantages. Each molecular method has different kit storage requirements. The Solana assays could be stored at 4 °C, the Simplexa at −20 °C and the Aries at room temperature. The Aries therefore is most advantageous in terms of storage as it reduces the freezer and refrigerator space required. The Solana is unique from the other molecular methods in that it also detects VZV, therefore having the potential to eliminate the need for separate VZV testing in the laboratory. However, the Solana does require a separate initial specimen lysis step prior to performing the amplification reaction, whereas the Simplexa and Aries assays are specimen-to-answer methods, with no additional specimen extraction step needed. Finally, as noted above, the Simplexa is the only method that allowed for testing of CSF specimens, although we did not investigate its performance with CSF specimens in this study.

There are some limitations to this study that should be noted. For logistical reasons, specimens were frozen prior to testing. However, in normal usage, specimens would not be frozen. The effect of prior freezing on our results is unknown. Secondly, the number of HSV-2 positive specimens was relatively low compared to the number of HSV-1 positive specimens in our patient population, so an evaluation of these assays with a larger numbers of HSV-2 specimens in the future would be worthwhile. Thirdly, the commercial transport medium used has not previously been studied with the molecular assays evaluated in this study. However, given the significantly greater detection rate with the molecular methods over culture, viral DNA appears to have been adequately preserved. Finally, we did not calculate the limits of detection for the assays evaluated. Since we had a limited supply of tests

available, we chose to focus on evaluating the performance of the three assays using clinical specimens, as we felt clinical sensitivity and specificity were more important performance indicators than the limits of detection.

5. Conclusions

All the molecular methods studied provided a significantly higher sensitivity than culture, and had a turnaround time of approximately 1–2 hours compared to days for culture results. Use of any of the three molecular methods studied could therefore lead to improved patient care.

Author contributions

The study was conceptualized by RS, NS, KR, and MD. Study data was acquired by KA and RS. Manuscript was initially drafted by RS and KA and revised by NS, KR, and MD. All authors approved the final version.

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Ethical approval

Study was approved by the Children's Hospital of Eastern Ontario Research Ethics Board: protocol CHEO 18/63 × .

Competing interest

None of the authors have any competing interests related to the manuscript.

Conflicts of interest

None.

Data statement

Data from the study is available upon reasonable request.

CRediT authorship contribution statement

Robert Slinger: Conceptualization, Investigation, Formal analysis, Methodology, Writing - original draft. **Kelly Amrud:** Investigation, Methodology, Formal analysis, Writing - original draft. **Nadia Sant:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Karam Ramotar:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Marc Desjardins:** Conceptualization, Investigation, Methodology, Writing - review & editing.

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