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Bayesian Evaluation of Solana HSV 1+2/VZV Assay Compared to Viral Culture and Commercial PCR Assay for Cutaneous or Mucocutaneous Specimens

Christine Arsenault,[a,b] Félix Camirand Lemyre,[c,d] Philippe Martin,[a,e] Simon Lévesque[a,e]

[a]Département de microbiologie et d'infectiologie, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada
[b]Hôpital du Sacré-Coeur de Montréal, Montréal, Québec, Canada
[c]Département de mathématiques, Faculté des sciences, Université de Sherbrooke, Sherbrooke, Québec, Canada
[d]Centre de recherche du CHUS, Sherbrooke, Québec, Canada
[e]Département de biologie médicale, CIUSSS de l'Estrie-CHUS, Sherbrooke, Québec, Canada

Christine Arsenault and Félix Camirand Lemyre contributed equally to this work and are named in alphabetical order.

ABSTRACT Results from the Solana HSV 1+2/VZV assay for the detection of herpes simplex virus 1 (HSV-1), HSV-2, and varicella-zoster virus (VZV) in cutaneous or mucocutaneous specimens were compared with that of viral culture and a commercial PCR assay (RealStar alpha herpesvirus PCR kit). Three hundred two mucocutaneous specimens, for which HSV-1, HSV-2, or VZV viral culture or PCR detection have been requested, were randomly selected and prospectively processed on the Solana assay and viral culture or the RealStar assay. Discordant results between culture and the Solana assay were further analyzed using the RealStar assay. A Bayesian latent class model was developed to estimate the performance of each method. Viral culture detected 123 positive specimens (85 HSV-1, 36 HSV-2, and 2 VZV), while the Solana assay detected 27 additional positive specimens (4 HSV-1, 11 HSV-2, and 12 VZV), in agreement with the RealStar PCR assay. The estimated sensitivity of the Solana assay according to our model was 92.7% to 98.7%, 87.1% to 97.8%, and 94.9% to 98.8% (95% confidence interval [CI]) for HSV-1 HSV-2, and VZV, respectively, while the estimated sensitivity of viral culture was 85.2% to 95.0%, 73.6% to 89.6%, and 30.9% to 45.8% (95% CI), respectively. A nonsignificant tendency toward increased sensitivity was noted for the Solana assay compared with culture for HSV-1 and HSV-2, and the Solana assay was significantly more sensitive than culture for the detection of VZV. The Solana assay performed comparably to the RealStar assay. Processing time was reduced with the Solana assay compared with viral culture.

KEYWORDS Solana, herpes simplex virus, varicella-zoster virus

Herpes simplex virus 1 (HSV-1), HSV-2, and varicella-zoster virus (VZV) are ubiquitous herpesviruses encountered by most humans during their lifetimes, presenting as mucocutaneous or disseminated illnesses when symptomatic. VZV causes chickenpox among children and reactivates as shingles in later periods of life (1) and can be complicated by bacterial infections or invasive diseases, such as encephalitis or myelitis. Herpetic cutaneous manifestations caused by HSV are generally recurrent. The most common manifestations of HSV-1 include herpes labialis, and both HSV-1 and HSV-2 cause herpes genitalis, with fewer recurrences with HSV-1 (2). Most people seropositive with HSV-2 do not present with classic symptoms and still can shed virus (3, 4). Various other presentations of HSV-1 and HSV-2 can occur, ranging from encephalitis to neonatal invasive infections. Although the clinical presentation of initial infection and reactivation of those viruses can be classic, atypical lesions occur and must be differ-

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Address correspondence to Christine Arsenault, christine.arsenault@usherbrooke.ca.

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entiated from other illnesses (5) and one virus from the others (6). Requirement for patient isolation and pharmacologic therapy depend on the specific viral etiology and the clinical presentation. These considerations warrant the need for a reliable and sensitive assay that settles diagnostic certainty in a timely manner. Such an assay would also facilitate decision-making regarding further diagnostic tests and the continuation of an empirical antiviral treatment.

Virus culture and immunofluorescence assays (IFAs) routinely used for diagnostic purposes in this context are plagued with poor sensitivity, and culture has a poor processing time, especially when it comes to VZV. Nucleic acid amplification tests (NAATs) are now recognized as the new gold standard because of their high sensitivity. Multiple nucleic acid amplification assays have been developed and evaluated for the detection of HSV or VZV in cutaneous or mucocutaneous specimens (6–9); however, most of the commercially available tests focus only on the detection of HSV. There is currently only one commercially available assay approved by both the FDA and Health Canada for the detection of HSV or VZV, including the differentiation of HSV-1 and HSV-2. The Solana HSV 1+2/VZV assay (Quidel, San Diego, CA) is a simple and, yet, promising multiplexed assay developed for the diagnosis of mucocutaneous lesions, and an assessment of its diagnostic accuracy is, therefore, needed.

Using a statistical approach called discrepant analysis, recent studies have estimated sensitivities for the detection of both viruses to range between 95% and 100%, with a specificity of 100% (10, 11). Discrepant analysis is a method that has been widely applied to assess diagnostic test performance when no perfect tests (i.e., 100% sensitivity and 100% specificity) are available. It consists of testing each sample with both the candidate test and a reference method, and if the results of the two tests disagree, then a resolver test is used to solve the discrepancy. However, this technique has been criticized by many for overestimating the sensitivity of a NAAT when it is compared with another assay plagued with poor sensitivity (12, 13). The purpose of this study is to evaluate the performance of the Solana HSV 1+2/VZV assay using a Bayesian latent class model. This statistical approach has increasingly gained acceptance as a valid alternative when diagnostic tests are assessed against an imperfect reference procedure (14). A particularity of these models is that throughout the estimation procedure, the true infection status is considered unknown (i.e., latent), which is arguably an adequate depiction of the reality of this assessment design. This study, thus, complements those of Nikolic (10) and Slinger (11) by pursuing a statistical analysis that allows the avoidance of the biases, if any, entailed by discrepant analysis.

MATERIALS AND METHODS

Study design. The HSV/VZV detection method used routinely in our laboratory is cell culture. Upon special request from a clinician, the RealStar assay is also available. We analyzed a total of 302 non-cerebrospinal fluid routine clinical specimens submitted to our laboratory for HSV and/or VZV detection. Specimens sent for viral culture by clinicians were collected randomly and prospectively from June to October 2018. Specimens sent for NAAT by clinicians were collected prospectively during the same period, but in our hospital, they are generally sampled from lesions judged visually to be of poorer diagnostic quality (for example, crusted lesions). Specimens were collected on swabs and transported in commercial universal transport medium (UTM) or homemade transport medium. Among the 302 specimens analyzed, 247 were initially analyzed by cell culture (230 for HSV-1 and HSV-2 detection and 17 for the detection of all three viruses) and 55 by the RealStar assay. The original specimen was then frozen at −80°C and processed on the Solana assay in batches between June and October 2018.

Cell culture method. Cell culture is processed on weekdays on MRC-5 cells in alveolated plates and incubated until cytopathogenic effect is noted, up to 1 week for HSV-1 and HSV-2 detection and up to 3 weeks for combined HSV-1, HSV-2, and VZV detection. The shell vial method is performed on MRC-5 cell lines in parallel only for VZV detection. Confirmation of virus type is done with SimulFluor HSV1/2 (Light Diagnostics; Sigma-Aldrich) or Merifluor VZV (Meridian Bioscience) immunofluorescent stain.

RealStar assay. The RealStar alpha herpesvirus PCR kit (Altona Diagnostics) is a multiplex PCR assay simultaneously detecting HSV-1, HSV-2, VZV, and an internal control target. It requires separate DNA extraction and amplification steps. DNA extraction is performed on an EasyMag platform (bioMérieux), and the PCR is run on the LC480II system (Roche) according to the manufacturer's instructions. This PCR kit is not approved by the FDA or Health Canada.

TABLE 1 Results of the 230 clinical specimens analyzed by cell culture and Solana assay for the diagnosis of HSV-1 and HSV-2

HSV-1/2 viral culture result[a]	No. of isolates with Solana assay result:				
	HSV-1 positive	HSV-2 positive	VZV positive	Negative	Total (no.)
HSV-1 positive	84	1	0	0	85
HSV-2 positive	0	36	0	0	36
Negative	4	10	6	89	109
Total	88	47	6	89	230

[a]HSV-1/2 viral culture incubated for a maximum of 1 week for a negative specimen, allowing recovery of HSV-1 and HSV-2 but not VZV.

Solana HSV 1+2/VZV assay. The Solana HSV 1+2/VZV assay is a helicase-dependent, isothermal-multiplexed NAAT. It simultaneously amplifies and detects the presence of HSV-1, HSV-2, and VZV viral DNA in a clinical sample. A competitive process control target is also amplified and detected, in order to validate each assay (monitor for inhibitory substances, reagent failure, and instrument failure). The assay was performed on the 302 thawed clinical samples according to the manufacturer's instructions.

Data collection and statistical analysis. Specimens for which discordant results were obtained between cell culture and Solana assay were processed on a third assay, the RealStar alpha Herpesvirus PCR kit. Sensitivity and specificity were estimated by means of a Bayesian latent class model based on the cross-classified results of the assays under examination, after their application in multiple populations (15). Bayesian credible intervals were generated using a hybrid Metropolis-Hastings/Gibbs algorithm. Details regarding the model and the credible interval calculations are provided below and in the Appendix.

Bayesian latent class model. A crucial consideration in the assessment of diagnostic assays is the assumption of conditional independence between tests, given the true disease status (16). Following the recommendation by Kostoulas (14), dependence terms with minimal impact on the final estimates were dropped and conditional dependencies were assumed only between sensitivities and specificities of the Solana HSV 1+2/VZV and the RealStar assay since both procedures rely on the same biological principle. The Bayesian latent class model developed by Black (15) allows for correlation between tests and was utilized to consider dependence between two assays, which are in our case the Solana and the RealStar assays. The cell culture diagnostic variables were integrated using the conditionally independent test model introduced in Joseph (17). The full specification of the resulting approach is described in broader details in the Appendix.

As is typically the case in Bayesian analyses, parameters involved in this model include the selection of prior distributions. Specifically, for each infection, a range of plausible values (RPVs) was assigned to each of the unknown parameters of the model, i.e., the sensitivity and specificity of each assay under consideration, as well as the prevalence range. For the assay accuracy parameters, the RPVs were formed by using the highest and lowest values of reported sensitivity and specificity in articles evaluating similar technologies as ours, as determined by a literature review from a panel of experts from the Centre Hospitalier Universitaire de Sherbrooke (2, 8–10, 18–20). The infection prevalence RPVs were extrapolated from the percentage of positive specimens identified from our laboratory in the past 3 years. Then, to each of these parameters and from their identified RPVs, a beta prior distribution was assigned by matching the center of its associated range with the mean of the beta distribution and by minimizing the squared distance between the endpoints of the range and the quantiles of order of 2.5% and 97.5% of the beta distribution.

To perform the calculation of the 95% equal-tailed credible interval estimation for the specificities and sensitivities of the examined assays, 290,000 iterations of a hybrid of Metropolis-Hastings/Gibbs update were used (see, e.g., Black [15]), after burn-in of 10,000 iterations. The algorithm was run in R 3.5.3 (The R Foundation for Statistical Computing, Vienna, Austria). Convergence of model runs was assessed by means of inspection of history plots of the parameters.

RESULTS

The results of the Solana assay compared with cell culture are described in Table 1 and 2. Among the 247 specimens analyzed, cell culture detected a total of 123 positive specimens (85 HSV-1, 36 HSV-2, and 2 VZV). Compared with culture, the Solana assay detected 27 additional positive specimens (4 HSV-1, 11 HSV-2, and 12 VZV). These positive results agreed with the results obtained with the RealStar PCR assay. Of note, 6 of the 12 VZV-positive samples by the Solana assay have not been cultured for VZV detection since the clinician requested only HSV detection. In addition, one specimen positive for HSV-2 by the Solana assay was initially identified as positive for HSV-1 by viral culture; however, the RealStar PCR assay result was concordant with the HSV-2 result obtained by the Solana assay, and the specimen was, therefore, considered positive for HSV-2 for the purpose of this study.

Results of the performance of the Solana assay compared with the RealStar PCR

TABLE 2 Results of the 17 clinical specimens analyzed by cell culture and Solana assay for the diagnosis of HSV-1, HSV-2, and VZV

HSV-1/2 and VZV viral culture[a]	No. of isolates with Solana assay result:				
	HSV-1 positive	HSV-2 positive	VZV positive	Negative	Total (no.)
HSV-1 positive	0	0	0	0	0
HSV-2 positive	0	0	0	0	0
VZV positive	0	0	2	0	2
Negative	0	1	6	8	15
Total	0	1	8	8	17

[a]Viral culture incubated for a maximum of 3 weeks for a negative specimen, allowing recovery of HSV-1, HSV-2, and VZV.

assay are described in Table 3. Among the 55 specimens analyzed, the RealStar PCR assay detected a total of 30 positive specimens (1 HSV-1, 9 HSV-2, and 20 VZV). One each of the HSV-1- and VZV-positive samples by the RealStar PCR assay was not detected by the Solana assay. During the entire study, no invalid results were obtained with the Solana assay and no sample contained both HSV and VZV viruses.

The Bayesian model described above was applied to estimate credible intervals for sensitivity and specificity of the Solana assay compared with viral culture, as shown in Table 4. The small number of specimens tested on both the Solana and the RealStar assays precluded the application of the model on these data.

DISCUSSION

The diagnosis of HSV infection based on clinical characteristics lacks both sensitivity and specificity in immunocompetent and immunocompromised populations. Before the development of molecular detection methods, viral culture was considered the gold standard method for the detection of HSV and VZV. As described in previous studies (6, 7, 21), the introduction of a NAAT represents an improvement compared with culture in terms of sensitivity. In our study, the Solana assay detected all the positive samples found by viral culture. Previous publications showed that sensitivity for the detection of HSV-1 and HSV-2 improved in an 18% to 24% range when the Solana assay was used in place of viral culture, and the magnitude of the gain is mostly influenced by the sensitivity of the viral culture since the Solana assay was invariably highly sensitive in a 95% to 100% range (10, 11). In our study, while a tendency toward an increased sensitivity of the Solana assay compared with culture was not statistically significant for HSV-1 and HSV-2 according to our Bayesian model, the detection of VZV was significantly higher.

One positive specimen by culture was revised as a possible false positive after being tested negative by the Solana assay and the RealStar PCR assay. The specimen was submitted a second time for culture and was negative. Cross-contamination was the presumed cause of the false positive since HSV and VZV were cultured on alveolated plates with multiple wells. These are vulnerable to the contamination of negative samples from the neighboring positives. In contrast, no suspicious positive result was obtained with the Solana assay.

The performance of helicase-dependent amplification (HDA) assay compared with PCR is usually good for HSV detection, according to previously published studies (22,

TABLE 3 Results of the 55 clinical specimens analyzed by RealStar PCR assay and Solana assay

RealStar PCR assay result	No. of isolates with Solana assay result:				
	HSV-1 positive	HSV-2 positive	VZV positive	Negative	Total
HSV-1 positive	1	0	0	0	1
HSV-2 positive	0	9	0	1	10
VZV positive	0	0	20	1	21
Negative	0	0	0	23	23
Total	1	9	20	25	55

TABLE 4 Sensitivity and specificity of the Solana assay compared with viral culture according to our Bayesian model

	Solana assay results		Viral culture results	
Virus name	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
HSV-1	92.7–98.7	94.2–98.7	85.2–95.0	95.2–99.0
HSV-2	87.1–97.8	91.4–97.3	73.6–89.6	95.2–98.9
VZV	94.9–98.8	91.4–97.3	30.9–45.8	94.8–98.2

23). In our study, the Solana assay performed equivalently to the RealStar PCR assay. The two positive samples found by the RealStar PCR assay and not by the Solana assay could be explained by the detection limit difference between the two assays. In the Solana assay, the clinical sample is diluted in the sample process buffer for the lysis step before the HDA analysis. In contrast, the clinical sample is concentrated during the EasyMag DNA extraction step before the PCR step. Finally, the two missed positive samples by the Solana assay were weakly positive according to the RealStar PCR assay (cycle threshold of 31 and 35). The dilution of the specimen in the Solana assay procedure is a downside of the simplicity and minimal processing time it requires.

We observed a significantly reduced processing time to obtain the results between the Solana assay and viral culture. Up to 12 samples can be processed on the Solana within 1 hour, and we batch specimens to process between 1 and 2 runs per day, so same-day or next-day results are the standard for specimens collected in our hospital. Cell culture results can take up to 1 week for HSV-1 and HSV-2 culture and 3 weeks for VZV culture. Even if the shell vial technique was used in parallel for the detection of VZV, the Solana assay was found to be much faster. The limited technical skills required to process a specimen on the Solana assay compared with viral culture also facilitates its integration in the workflow. Timely and accurate diagnostic laboratory tests are necessary for instituting appropriate therapeutic management and making decisions regarding intrapartum delivery, as well as for isolation purposes (6). Prompt and precise laboratory testing is also useful for ruling out alternative diagnostics, particularly in atypical presentation of the disease or in immunocompromised patients (24). Finally, determining the infecting virus type is useful for prognostic purposes since recurrent manifestations are less common with genital HSV-1 than with HSV-2 (25).

This study has some limitations. First, samples had to be frozen between each assay, which may have altered the quality of the sample in an uneven way and reduced the sensitivity of the Solana assay, which was done last in most cases. Second, an analysis of the sensitivity of an assay which is expected to be highly sensitive compared with a less sensitive one is a difficult task because it is possible to overestimate the sensitivity of the evaluated assay. While previous studies have addressed the problem by defining a criterion for a real positive specimen and used discrepant analysis for disagreement resolution, we innovated by using a Bayesian latent class model to estimate performance characteristics of the assay. Although the Bayesian model considered in this work avoids the potentially biased setting inherent to discrepant analysis, where the test results under consideration are implicitly incorporated in the definition of the true infection status, the estimation procedure relies on a choice for the prior distributions, which involves a certain degree of subjectivity. These prior distributions are crucial to achieve estimation in our context where, in the absence of a gold standard, the true infection status is unknown. While the state-of-the-art practice is to base prior elicitation on scientifically justifiable information unrelated to the data at hand, which may include opinions of experts (14), it is also well recognized that their selection generally influences the data analysis. To evaluate the extent of the possible bias that could have been related to the choice of the prior distributions, we ran sensitivity analyses involving different sets of Beta prior distributions. Details can be found in the Appendix. While these numerical investigations suggest that our model allows for a relatively

robust estimation of the Solana sensitivity parameters, the width of the credible intervals associated with the Solana specificity parameters seems to be slightly more affected by less informative priors. However, given that NAATs are generally considered highly specific, considering larger ranges of plausible values for these parameters is arguably not necessary in our context.

Finally, it should be noted that while the specimens for viral culture were collected randomly, the specimens for which a NAAT was requested were sampled in specific clinical contexts, such as VZV suspicion or crusted lesions, which explains why VZV is overrepresented in this subgroup of specimens.

Conclusion. In summary, the Solana HSV 1+2/VZV assay detects more positive samples than viral culture; is more sensitive than viral culture for the diagnosis of VZV; and performs comparably to the RealStar assay for the detection of HSV-1, HSV-2, and VZV in cutaneous or mucocutaneous specimens. Finally, processing time is reduced by the use of the Solana HSV 1+2/VZV assay.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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