Comparison of Simplexa HSV 1 & 2 PCR with Culture, Immunofluorescence, and Laboratory-Developed TaqMan PCR for Detection of Herpes Simplex Virus in Swab Specimens

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The Simplexa HSV 1 & 2 direct PCR assay was compared with conventional cell culture, cytopsin-enhanced direct fluorescent antibody (DFA), and a laboratory-developed real-time TaqMan PCR (LDT HSV PCR) using extracted nucleic acid for the detection of herpes simplex virus (HSV) in dermal, genital, mouth, ocular, and other swab samples. One hundred seventy-one swabs were tested prospectively, and 58 were positive for HSV (34 HSV-1 and 24 HSV-2). Cytopsin-DFA detected 50 (86.2%), conventional cell culture 51 (87.9%), Simplexa direct 55 (94.8%), and LDT HSV PCR 57 (98.3%) of 58 true positives. Simplexa direct detected more positives than DFA and culture, but the differences were not significant \((P = 0.0736 \text{ and } P = 0.3711, \text{ respectively, by the McNemar test})\). Samples that were positive by all methods \((n = 48)\) were strong positives (LDT cycle threshold \([C_T]\) value, 14.4 to 26.1). One strongly positive sample was falsely negative by LDT HSV PCR due to a failure of TaqMan probe binding. Three samples falsely negative by Simplexa direct had high \(C_T\) values by LDT HSV PCR (LDT \(C_T\), 35.8 to 38.2). Omission of the DNA extraction step by Simplexa direct led to a drop in sensitivity compared to the sensitivity of LDT HSV PCR using extracted samples (94.8% versus 98.3%, respectively), but the difference was not significant \((P = 0.6171)\). Simplexa HSV 1 & 2 direct PCR was the most expensive but required the least training of the assays used, had the lowest hands-on time and fastest assay time (75 min, versus 3 h by LDT HSV PCR), and provided the HSV type.

Herpes simplex virus (HSV) causes a wide spectrum of clinical manifestations. Although lesions are usually self-limited, severe disease can occur, particularly in compromised hosts, pregnant women, and neonates. The diagnosis and treatment of HSV eye infections is crucial to protect vision, while the recognition of genital herpes is important to prevent transmission and provide counseling. Thus, rapid and accurate laboratory diagnosis is important to guide management and institute appropriate therapy.

Laboratory diagnosis of herpetic lesions has relied on culture of infectious virus or direct fluorescent antibody (DFA) staining of infected cells. However, these methods are labor intensive, require highly skilled personnel, rely on subjective interpretation, and are more adversely impacted by collection technique, transport conditions, and delays in processing than DNA amplification methods.

While previous reports have shown that laboratory-developed PCR assays are more sensitive than culture for the diagnosis of HSV in dermal and genital samples (1–11), no prior studies have analyzed the performance characteristics of the Simplexa HSV 1 & 2 direct PCR assay (Focus Diagnostics, Cypress, CA), a new commercial assay that can be performed directly on clinical samples in a nonmolecular laboratory.

The objective of this study was to compare the performance, time to result, and cost of the Simplexa HSV 1 & 2 direct PCR with those of conventional cell culture, DFA, and a laboratory-developed real-time TaqMan PCR (LDT HSV PCR) for the detection of HSV in dermal, genital, ocular, mouth, or other swab samples.

MATERIALS AND METHODS

Clinical specimens. Swab specimens \((n = 171)\) submitted to the Clinical Virology Laboratory for HSV DFA and/or culture from November 2012 to February 2013 were tested prospectively. All swabs were included on study days when staffing permitted participation. Swabs were collected from local clinics, doctors’ offices, and inpatient wards in 3 ml of M4 viral transport medium (VTM) (Remel, Lenexa, KS) and processed for both DFA and culture within 2 to 8 h of collection. Samples were then coded and frozen at \(-70°C\) until tested within 1 to 4 weeks by both Simplexa and LDT HSV PCR.

Virus isolation. Samples were inoculated into A549 and MRC5 cells in roller tubes, 0.2 ml per tube, and absorbed for 1 h at 37°C. The medium was replaced, and tubes were incubated at 37°C. Cultures were examined daily for cytopathic effects (CPE) for 7 days for HSV and 14 days for varicella-zoster virus (VZV). HSV isolates were typed using D3 DFA reagent (Diagnostic Hybrids, Athens, OH).

Cytopsin-enhanced DFA. Samples were centrifuged to pellet cells as previously described (12). Cell pellets were applied to slides using a cytopsin and then fixed and stained with SimulFluor HSV/VZV reagent (Millipore, Billerica, MA). Slides were examined using a fluorescence microscope and, as recommended by the manufacturer, at least one cell showing the expected pattern of fluorescence was required for a positive result.

Simplexa HSV 1 & 2 direct PCR. Simplexa HSV 1 & 2 PCR reagents were sold as analyte-specific reagents (ASR). Coded specimens were thawed, vortexed, and pulse spun. To each well of the 96-well disc, the following reagents were added: first, 6 μl of master mix, 0.4 μl of prepared HSV-1/-2 master mix, composed of 4 μl of master mix, 0.4 μl each of HSV-1 and HSV-2 primers, 0.2 μl of internal control primer, 0.2 μl of internal control DNA, and 0.8 μl of nuclease-free water; and second, 4 μl of unextracted patient...
specimen or positive- or negative-control material. The discs were sealed and placed in a 3M integrated cycler. Amplification was performed for 40 cycles according to the manufacturer’s recommendations.

**LDT HSV real-time TaqMan PCR.** The LDT HSV type-common TaqMan PCR, targeting the POL gene (LDT HSV PCR) and using published primers and probe, was performed as the primary diagnostic test per routine clinical practice (13). Samples that were untyped (HSV culture negative) or with discordant PCR results were also tested by a clinically validated type-specific TaqMan PCR targeting the HSV-1 and HSV-2 gG genes (LDT HSV-typing PCR) (14). DNA was extracted from 200 μl of sample and eluted in 55 μl on an EasyMag instrument (bioMérieux, Durham, NC), and 5 μl of extract was added to ABI universal master mix. Amplification was performed for 45 cycles on an ABI 7500 instrument.

**Data analysis.** Positive results were accepted as true when obtained by two or more methods. Statistical analysis was performed using McNemer’s test. A P value of <0.05 was considered significant. Figures were constructed using GraphPad Prism version 5.0 (GraphPad Software).

**RESULTS**

One hundred seventy-one samples were included in the study. Patient demographics included 129 outpatients, 31 inpatients, and 11 patients whose location was not specified, 126 female and 23 pediatric patients (age range, 1 day to 95 years). Seventy-four swabs (43.4%) were from male patients, and 148 adult and 23 pediatric patients (age

<table>
<thead>
<tr>
<th>Test method</th>
<th>Total no. positive (n = 58)</th>
<th>No. true positive</th>
<th>Sensitivity [% (95% CI)]</th>
<th>Specificity [% (95% CI)]</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytospin-DFA</td>
<td>50</td>
<td>50</td>
<td>86.2 (0.748–0.931)</td>
<td>100 (0.995–1.000)</td>
<td>100 (0.915–1.000)</td>
<td>93.4 (0.873–0.968)</td>
</tr>
<tr>
<td>Conventional culture</td>
<td>52</td>
<td>51</td>
<td>87.9 (0.768–0.943)</td>
<td>99.1 (0.945–1.000)</td>
<td>98.1 (0.889–1.000)</td>
<td>94.1 (0.882–0.973)</td>
</tr>
<tr>
<td>Simplexa direct PCR (no extraction)</td>
<td>55</td>
<td>55</td>
<td>94.8 (0.853–0.988)</td>
<td>100 (0.995–1.000)</td>
<td>100 (0.922–1.000)</td>
<td>97.4 (0.923–0.995)</td>
</tr>
<tr>
<td>LDT HSV PCR (with sample extraction)</td>
<td>57</td>
<td>57</td>
<td>98.3 (0.900–1.000)</td>
<td>100 (0.995–1.000)</td>
<td>100 (0.925–1.000)</td>
<td>99.1 (0.947–1.000)</td>
</tr>
</tbody>
</table>

*CI, confidence interval.

a Simplexa detected more positives than DFA, but the difference was not significant (P = 0.0736).

b Simplexa detected more positives than culture, but the difference was not significant (P = 0.3711).

c Simplexa direct without extraction failed to detect 3 samples with very low viral loads, but the difference between Simplexa and LDT HSV PCR was not significant (P = 0.6171).

d LDT HSV PCR missed one true positive due to failure of probe binding.

\[VZV\] was detected in 8 samples by culture and/or DFA; VZV PCR was not done.

When analyzed by site, 30/74 (40.5%) known genital samples were positive: 24/30 (80%) by DFA, 25/30 (83.3%) by culture, 28/30 (98.8%) by Simplexa, and 30/30 (100%) by LDT HSV PCR. When nongenital and unspecified sites were combined, 28/97 (28.9%) were positive: 26/28 (92.9%) by DFA and culture, and 27/28 (96.4%) by Simplexa and LDT HSV PCR. Although PCR tests showed a greater advantage over DFA and culture for genital samples, the difference did not reach significance due to low sample size.

For samples positive by both PCR tests, Simplexa direct PCR (unextracted) had a higher mean cycle threshold (C<sub>T</sub>) value than LDT HSV PCR using extracted samples (C<sub>T</sub> 24.9 versus 20.9). As shown in Fig. 1, samples positive by all methods (48 of 58, 82.8%) were strong positives (LDT C<sub>T</sub> 14.4 to 26.1). DFA detected samples with LDT C<sub>T</sub> values of <26. Culture detected samples with LDT C<sub>T</sub> values of <28. Simplexa direct detected samples with LDT C<sub>T</sub> values of <35.8. For samples positive by both PCR tests, the mean C<sub>T</sub> values were higher for HSV-1 (19.9 with LDT and 23.6 with Simplexa) than for HSV-2 (22.6 with LDT and 27 with Simplexa). Details of discordant samples are shown in Table 2. Eight were

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**FIG 1** PCR cycle threshold (C<sub>T</sub>) values for HSV-positive samples detected by various test methods (D, DFA; C, culture; S, Simplexa PCR; L, LDT HSV PCR).
HSV-2, and 3 were HSV-1. One sample that was negative on initial and repeat testing by LDT HSV PCR was positive by all three of the other methods, including Simplexa direct PCR, which had a $C_T$ value of 24.9 (Table 2). Gel electrophoresis demonstrated that LDT HSV PCR generated an amplicon of the expected size. However, sequencing the amplicon revealed four point mutations in the probe-binding site (R. Hodinka, personal communication). Thus, it appears that the LDT TaqMan probe failed to bind to the HSV type-common POL region. Of note, the LDT HSV-typing PCR detected this specimen.

Another sample that was positive at 3 days by culture alone was a false positive. When the laboratory worksheets were reviewed, it was found that 3 laboratory specimens were contaminated during subpassage on that day, of which two were recognized and not reported. However, this sample was inadvertently reported as positive. The original sample was retested and found negative by all methods. Three samples that were false negative by Simplexa direct but positive by LDT HSV PCR demonstrated low viral loads ($C_T$, 35.8 to 38.2).

The materials, reagents, and labor costs per reportable result, as well as the technical expertise required, are shown in Table 3. The reagent costs for the Simplexa direct, quoted based on anticipated test volume, were the highest ($39.54) and included type-specific primers and internal control. The LDT HSV PCR included extraction reagents ($9.51 per sample) and testing of all positive samples (34% of total samples) by type-specific PCR. Both Simplexa and LDT HSV PCR costs were based on 5 patient samples per run, whereas the typing PCR cost was based on 2 patient samples per run, plus controls. Due to its minimal hands-on time, Simplexa had the lowest estimated labor costs ($2.69) and required the lowest skill level.

**DISCUSSION**

Real-time PCR has allowed a limited number of clinical laboratories with significant molecular expertise to implement laboratory-developed tests for viral diagnosis. Although PCR for detection of HSV in spinal fluid quickly replaced culture of brain biopsy tissue as the standard of care, DFA and cell culture are still commonly used for detection of HSV in swab samples in hospital laboratories. The reasons include the lack of user-friendly commercial PCR kits, the need to purchase expensive molecular equipment and, in our case, concerns about contaminating spinal fluid samples with HSV from lesion swabs that often contain significant amounts of virus.

In this study, we evaluated a new commercially available test, Simplexa HSV 1 & 2 direct, for the detection and typing of HSV in swab samples. Simplexa HSV 1 & 2 direct PCR detected 94.8% of true positives, more than DFA (86.2%) or culture (87.9%). Compared to DFA, culture, and a laboratory-developed TaqMan PCR, Simplexa HSV direct was simpler to perform and required less staff training and expertise. In addition, at 75 min from start to finish, Simplexa had the shortest assay time. Because it eliminated the nucleic acid extraction step and utilized a simple closed-tube format and a compact cycler, Simplexa could be conveniently performed in the same area as DFA and cell culture and did not require molecular laboratory space. Simplexa HSV direct also

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**TABLE 2 Details of discordant samples**

<table>
<thead>
<tr>
<th>Sample source</th>
<th>DFA result</th>
<th>Culture result</th>
<th>Day of positive culture result</th>
<th>Simplexa PCR result</th>
<th>Simplexa $C_T$ value</th>
<th>LDT HSV PCR result</th>
<th>LDT HSV-typing PCR result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buttocks</td>
<td>HSV</td>
<td>HSV-2</td>
<td>1</td>
<td>HSV-2</td>
<td>24.9</td>
<td>Negative</td>
<td>&gt;45</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Labia</td>
<td>Negative</td>
<td>HSV-1</td>
<td>3</td>
<td>HSV-1</td>
<td>28.4</td>
<td>HSV</td>
<td>26.4</td>
<td>Not done</td>
</tr>
<tr>
<td>Vulva</td>
<td>Negative</td>
<td>HSV-2</td>
<td>2</td>
<td>HSV-2</td>
<td>31.5</td>
<td>HSV</td>
<td>27.6</td>
<td>Not done</td>
</tr>
<tr>
<td>Vulva</td>
<td>HSV (1 cell)</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>34.2</td>
<td>HSV</td>
<td>28.2</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Genital</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>37.4</td>
<td>HSV</td>
<td>32.7</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Skin, NOS</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>37.7</td>
<td>HSV</td>
<td>34.8</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Labia</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>39.0</td>
<td>HSV</td>
<td>35.3</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Vaginal sore</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>&gt;40</td>
<td>HSV</td>
<td>35.8</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Perineal ulcer</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>&gt;40</td>
<td>HSV</td>
<td>37.7</td>
<td>HSV-2</td>
</tr>
<tr>
<td>Corneal ulcer</td>
<td>Inadequate</td>
<td>Negative</td>
<td>NA</td>
<td>HSV-2</td>
<td>&gt;40</td>
<td>HSV</td>
<td>38.2</td>
<td>HSV-1</td>
</tr>
<tr>
<td>Mouth</td>
<td>Negative</td>
<td>Positive</td>
<td>6</td>
<td>Negative</td>
<td>&gt;40</td>
<td>Negative</td>
<td>&gt;45</td>
<td>Not done</td>
</tr>
</tbody>
</table>

* NOS, not otherwise specified; NA, not applicable.

**TABLE 3 Comparison of estimated costs per reportable result**

<table>
<thead>
<tr>
<th>Test</th>
<th>Assay time</th>
<th>Frequency of testing</th>
<th>Cost ($)</th>
<th>Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Materials and reagents</td>
<td>Labor</td>
</tr>
<tr>
<td>Cytopsin-DFA</td>
<td>90 min</td>
<td>On demand</td>
<td>5.14</td>
<td>8.78</td>
</tr>
<tr>
<td>Conventional culture</td>
<td>1–7 days</td>
<td>Examined once a day</td>
<td>5.40</td>
<td>11.94</td>
</tr>
<tr>
<td>Simplexa direct PCR</td>
<td>75 min</td>
<td>Once a day</td>
<td>39.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.69</td>
</tr>
<tr>
<td>LDT HSV PCR with extraction</td>
<td>3 h</td>
<td>Once a day</td>
<td>19.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cost per reportable includes controls and is adjusted for run size. Purchase or rental of equipment is not included.

<sup>b</sup> Including thermal cycler rental in reagent costs will add $2 per test.

<sup>c</sup> Includes costs of a second typing PCR for samples positive by initial HSV type-common PCR.
saved 30 to 45 min in extraction time and reagent costs of $9 per sample and eliminated the need for an extraction instrument. However, it likely had lowered sensitivity, as the LDT HSV PCR utilizing extracted nucleic acid detected three low-titer positive samples (C_\text{T}> 35 to 38; mean of 37.2) that were negative by Simplexa. Preliminary studies using archived samples showed that LDT HSV PCR was similar in sensitivity to Simplexa PCR when extracted samples were tested by both tests but had a mean C_\text{T} value 2.2 cycles lower (i.e., more sensitive) than that of Simplexa direct PCR used on unextracted samples (authors’ unpublished data). Lower C_\text{T} values were also observed for extracted versus unextracted study samples. In addition to removing inhibitors, extraction steps commonly concentrate the sample 4- to 8-fold, thus enhancing sensitivity. A new version of Simplexa HSV 1 & 2 PCR that is in development uses a much larger sample input in an 8-well direct amplification disk and, thus, should have improved sensitivity.

Our results differed from those of previous studies that typically reported cell culture sensitivities of only 58 to 79% compared to the sensitivities of a variety of laboratory-developed real-time HSV PCR assays for dermal and genital samples (1–4, 7, 9). Studies with a greater number of eye, throat, or bronchiolar lavage fluid samples had lower culture-positive rates (7). One study with an unusually low culture-positive rate of 37% attributed it to poor-quality samples with low viral loads (11). In our study, culture detected 89.5% of LDT positives (P = 0.8888) and 92.7% of Simplexa direct positives (P = 0.3711). There were several unique aspects to our study that likely account for the better-than-expected culture results. Most importantly, all samples were collected locally and inoculated into culture on the day of collection, and thus, virus infectivity was preserved. In other reports, samples were transported a distance (1–3), sometimes under adverse conditions (11), or were frozen and thawed prior to inoculation (3). Most often, the time from collection to culture inoculation and the storage conditions were not stated. Additionally, our samples were adsorbed onto the cell monolayers, rotated, and read for 7 to 14 days, all of which enhance sensitivity. Thus, clinical settings to our study that likely account for the better-than-expected culture results. Most importantly, all samples were collected locally and inoculated into culture on the day of collection, and thus, virus infectivity was preserved. In other reports, samples were transported a distance (1–3), sometimes under adverse conditions (11), or were frozen and thawed prior to inoculation (3). Most often, the time from collection to culture inoculation and the storage conditions were not stated. Additionally, our samples were adsorbed onto the cell monolayers, rotated, and read for 7 to 14 days, all of which enhance sensitivity. Thus, clinical settings

Several prior studies have reported that HSV-1-positive samples were more likely to be culture positive, with rates of culture positivity as high as 88.8% compared to LDT PCR (5–7, 10). In our study, the mean C_\text{T} values were lower for HSV-1-positive than for HSV-2-positive samples, and of 51 culture-positive samples, 33 were HSV-1 and 18 HSV-2. Of 5 culture-negative samples, 1 was HSV-1 and 4 were HSV-2.

Some studies have documented the effect of lesion stage on virus titer and positive results, with skin vesicles harboring the most virus (4, 8). Unfortunately, we have no information on lesion stage in our patients. However, many studies, including ours, have linked C_\text{T} values and, thus, virus titer, to the ability to isolate virus in cell culture. In prior reports, culture-positive samples have had mean or median C_\text{T} values in the range of 20 to 26 and culture-negative samples had mean C_\text{T} values of 31 to 36 (1, 2, 5–7). Attesting to the high quality of samples in our study, 51 of 58 PCR-positive samples had C_\text{T} values of <28 and all were culture positive (mean C_\text{T}, 19.9). Only 6 of 58 PCR-positive samples had C_\text{T} values of >30, and all were culture negative (mean LDT C_\text{T}, 34.7). Thus, our study confirmed the value of PCR over culture when low titers of virus are present.

We could find no other studies that compared DFA to PCR for swab samples. DFA is best suited to on-site testing of low numbers of samples. DFA performed well in our study since the samples were of high quality and the cell pellets were concentrated and then cytospun onto slides, which improves cell yield and morphology (12). In our laboratory, DFA typically detects over 90% of HSV culture-positive skin lesions but only 80% of mucosal lesions. Studies that do not cytospin their samples and test mostly mouth swabs will have a much lower yield from DFA (15). Importantly, DFA is subjective, operator dependent, and requires a higher level of expertise and training than Simplexa direct PCR.

One of the strengths of the Simplexa HSV 1 & 2 direct PCR is that it allows for HSV type identification. The HSV type can suggest the mode of transmission or origin of infection, as well as predict the frequency of reactivation. As our gold standard screening test, we used a type-common POL gene LDT HSV PCR (13) that missed a sample with a high virus titer due to point mutations in the TaqMan probe binding site. Our LDT type-specific assay targeting the gG gene (14) detected this sample, however, as did Simplexa HSV direct PCR.

Finally, as more attention is being paid not only to performance but to cost effectiveness, this study addresses costs for the various tests. Other reports have noted that reagent costs are greater for laboratory-developed PCR than for culture but labor costs are less (4, 8). Our analysis confirmed this. While Simplexa PCR is significantly more expensive than LDT PCR, it offers convenience, HSV typing in the initial screen, and an internal control. Equipment cost is not included in most published reports, yet it is the rate-limiting step for many laboratories hoping to implement PCR. Importantly, Simplexa direct PCR does not require the purchase of expensive molecular instruments. A number of assays are now available on this platform, increasing its utility and lowering the per-test costs.

There were several limitations to our study. First, testing was performed on all samples received on study days, but due to staffing limitations, the study could not be performed every day during the 4-month study period; however, given that testing days occurred at random, this is unlikely to have introduced a selection bias. Second, charts were not available for review, so we were unable to correlate laboratory findings with clinical data, such as the stage of lesion or whether the patient had a history of HSV infection or was on therapy. Third, our study used the universal 96-well disk, which is suitable for high-throughput testing, but the wells hold a smaller sample volume. Focus Diagnostics has a new 8-well direct-amplification disk, which holds a larger volume of patient specimen. Whether this will improve sensitivity was not evaluated. Fourth, samples were frozen prior to testing by both PCR tests. Freezing and thawing could enhance the performance of Simplexa direct by partial lysis and removal of inhibitors. However, a limited study of 5 unextracted clinical samples comparing PCR results showed no effect: the mean C_\text{T} values were 22.5 un-frozen, 22.5 after 1 week at −70°C, and 22.0 after 3 weeks. Finally, since the majority of samples were submitted only for HSV, we chose not to increase test costs by routinely performing VZV PCR on all study samples. In general, VZV was clinically suspected for the samples positive for VZV by DFA or culture.

In summary, ours is the first study to analyze the performance characteristics of the commercially available Simplexa HSV 1 & 2...
direct PCR on swab samples. Simplexa HSV direct detected more positives than conventional methods, was simple to perform, incorporated an internal control, provided the HSV type, and was completed in 75 min. Simplexa direct PCR also utilized an instrument with a small footprint that can be leased via reagent rental. Testing can be performed outside the molecular laboratory and, thus, PCR of lesions can be performed separately from handling of cerebrospinal fluid samples, reducing the risk of carryover contamination.

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REFERENCES