Rapid and Direct Detection of Herpes Simplex Virus in Cerebrospinal Fluid

Using a Commercial Real-Time PCR Assay

Matthew J. Binnicker*, Mark J. Espy, and Cole L. Irish

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,
Mayo Clinic, Rochester, Minnesota 55905

* Corresponding author: Matthew J. Binnicker
Mayo Clinic
200 First Street SW, Hilton 454
Rochester, MN 55905
Phone: (507) 538-1640
Fax: (507) 284-4272
E-mail: binnicker.matthew@mayo.edu
ABSTRACT

Central nervous system infection due to herpes simplex virus (HSV) is a medical emergency and requires a rapid diagnosis and initiation of therapy. In this study, we compared a routine real-time PCR assay for HSV type 1 (HSV-1) and HSV-2 to a recently FDA-approved direct PCR assay (Simplexa™ HSV-1/2 Direct; Focus Diagnostics, Cypress, CA) using cerebrospinal fluid samples (n=100). The Simplexa HSV-1/2 assays demonstrated a combined sensitivity and specificity of 96.2% (50/52) and 97.9% (47/48), respectively. In addition, the Simplexa assay does not require nucleic acid extraction and results are available in 60 minutes.

SHORT-FORM PAPER

Herpes simplex viruses type 1 (HSV-1) and 2 (HSV-2) are a common cause of dermal, oral and genital infections worldwide. Following primary infection, HSV-1 and HSV-2 establish latency in the dorsal root ganglia and persist there for the life of the host. In some cases, primary infection or reactivation of the virus can lead to central nervous system (CNS) disease (1). HSV-1 is responsible for approximately 10% of all cases of encephalitis and is the most common cause of fatal sporadic viral encephalitis worldwide. The mortality rate of HSV-1 encephalitis (HSE) may exceed 70% if untreated, and greater than 95% of untreated survivors will suffer lifelong sequelae (2). Infection with HSV-2 may result in meningitis or meningoencephalitis, which may recur despite therapy (3). Neonatal infection with HSV-2 is especially devastating and disseminated disease may occur in approximately 25% of cases (4).

Due to the high morbidity and mortality associated with HSV infection of the CNS, it is important to establish a diagnosis and initiate therapy as soon as possible. Detection of HSV-1 and HSV-2 in cerebrospinal fluid (CSF) using real-time PCR is now recognized as the gold-standard approach for the diagnosis of HSE and herpes meningitis (5, 6). A number of
laboratory developed real-time PCR assays for the detection and differentiation of HSV-1/2 have been described with sensitivities and specificities typically exceeding 95% (7-10). These methods have demonstrated superior performance compared to routine viral cell culture and have decreased the turnaround time to <8 h in most situations. However, laboratory developed tests (LDTs) for the detection of HSV-1/2 in CSF lack standardization and require preanalytical nucleic acid extraction, which increases the turnaround time by up to several hours. Furthermore, the volume of CSF that is required for testing varies, and this can be an important factor in the diagnosis of neonatal HSV when the amount of specimen recovered is typically low.

Recently, the Food and Drug Administration (FDA) approved the first real-time PCR assay (Simplexa™ HSV-1/2 Direct; Focus Diagnostics, Cypress, CA) for the detection and differentiation of HSV-1/2 from CSF. This assay does not require up-front nucleic acid extraction, and results are available in approximately 60 min. In this study, we compared the performance of the Simplexa HSV-1/2 Direct assay to our routine real-time PCR (Roche HSV-1/2 analyte specific reagents [ASR]; Roche Diagnostics, Indianapolis, IN) using a selected panel of clinical CSF samples (n=100). Samples were submitted for routine testing by the Roche HSV-1/2 ASR on the LightCycler 2.0 (Roche) as previously described (11). This routine process includes preanalytic nucleic acid extraction on the MagNA Pure (Roche), which requires 200 µL of CSF. Following extraction, 5 µL of nucleic acid was tested by the Roche ASR and the results reported as “positive” or “negative” for HSV-1 and/or HSV-2 based on melting curve analysis. In addition, a result of “HSV detected – type indeterminate” is possible when a melting curve is identified that falls between the expected range for HSV-1 and HSV-2. A prior study analyzed samples showing “indeterminate” results, and using sequencing determined that HSV-1 or HSV-2 nucleic acid is present, but a 1 to 3-bp polymorphism in the probe region of the real-time PCR.
assay generates the abnormal melting curve result (11). Our internal validation of the Roche ASR demonstrated a limit of detection (LoD) in CSF of ~10 copies/µL. For this study, samples were selected following routine testing to ensure an adequate representation of positive (n=52) and negative (n=48) results. Among the 52 positive samples by our routine method, 37 (71.2%) were positive for HSV-2, 11 (21.2%) for HSV-1, and 4 (7.7%) were resulted as ‘HSV detected – type indeterminate’. The average crossing point (C_p) value of these positive CSF samples by the Roche ASR was 32.02 (range, 27.25 – 34.39). Following routine testing, samples were stored at 4°C and then tested in a blinded fashion by the Simplexa HSV-1/2 Direct assay within 48 h. Testing by the Simplexa assay was performed using the 3M Integrated Cycler (Focus) according to the manufacturer’s FDA-cleared package insert. The Simplexa HSV-1/2 assay requires 50 µL of raw CSF, which is pipetted directly into the supplied Direct Amplification Disc (Focus).

Samples showing discrepant results were stored at 4°C and tested within 24 h by a third real-time PCR assay (artus® HSV-1/2; Qiagen, Germantown, MD) (12) according to the manufacturer’s CE (European Conformity)-marked product insert with minor modifications. Briefly, nucleic acid extraction was performed using the MagNa Pure (Roche) and 5 µL of extract was combined with 15 µL mastermix (Qiagen) and tested on the LightCycler 2.0 (Roche). According to the manufacturer, the LoD of the artus HSV-1/2 assay is 1 copy/µL.

Following testing of 100 clinical CSF samples, the results of the Simplexa HSV-1/2 Direct assays were compared to those of our routine method (Roche HSV-1/2 ASR), which was established as the reference standard for this evaluation. The Simplexa HSV assays demonstrated a sensitivity of 100% (11/11) (95% confidence interval [CI], 70.0 - 100%) for HSV-1 and 100% (37/37) (95% CI, 88.8 - 100%) for HSV-2. Two samples were reported as
“HSV detected - type indeterminate” by our routine method but negative by the Simplexa HSV-1 and HSV-2 assays. The $C_p$ values for these two samples were 32.3 and 32.7 cycles by the Roche ASR. Interestingly, these two samples were negative for HSV-1/2 by the artus real-time PCR assay and were also negative upon repeat testing by the Roche ASR. The specificity of the Simplexa HSV-1 and HSV-2 assays was 100% (85/85) (95% CI 94.8 - 100%) and 98.3% (58/59) (95% CI, 90.2 – 99.9%), respectively. One sample was found to be positive for HSV-2 by the Simplexa assay, but negative by our routine method. This sample demonstrated a $C_p$ value of 40 by the Simplexa assay and was negative for HSV-2 by artus, suggesting the Simplexa result may have been falsely-positive.

In this study, the recently FDA-approved Simplexa HSV-1/2 Direct assay demonstrated a combined sensitivity and specificity of 96.2% (50/52) and 97.9% (47/48), respectively, when compared to the initial results of our routine method. Notably, the two samples that were negative by the Focus HSV-1/2 Direct assay but “HSV detected – type indeterminate” by our routine method were repeated by the Roche ASR and found to be negative. These samples were also negative by the artus HSV-1/2 real-time PCR, suggesting the initial results of our routine method may have been falsely positive. To our knowledge, this is the first report describing the performance of the Simplexa HSV-1/2 Direct CSF assay compared to routine real-time PCR. Importantly, the Simplexa HSV-1/2 Direct assay does not require prior nucleic acid extraction and reduced the overall turnaround time approximately 4-fold (~4 h by our routine method compared to ~1 h by the Simplexa assay). This may have a significant impact on the management of patients being evaluated for central nervous system disease as a result in ~ 1 h may allow for antimicrobial therapy to be rapidly adjusted. For example, a positive result for HSV-1/2 may prompt the discontinuation of antibiotic treatment, while a negative result may...
allow for acyclovir therapy to be discontinued and diagnostic testing focusing on other potential etiologies. The Simplexa assay requires only 50 µL of CSF, which may be important in certain cases, including the evaluation of neonatal CNS disease where the recovery of CSF is often limited.
117 References


### Table 1. Comparison of the Focus Simplexa HSV-1/2 direct assay to routine real-time PCR using cerebrospinal fluid samples (n=100)

<table>
<thead>
<tr>
<th>Focus HSV-1</th>
<th>HSV-1 Positive</th>
<th>HSV-1 Negative</th>
<th>HSV Type Indeterminate&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Negative</td>
<td>0</td>
<td>85</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
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<table>
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<th>Focus HSV-2</th>
<th>HSV-2 Positive</th>
<th>HSV-2 Negative</th>
<th>HSV Type Indeterminate</th>
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<td>Positive</td>
<td>37</td>
<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>58</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

ASR, analyte specific reagents; HSV-1/2, herpes simplex virus types 1 and 2

<sup>a</sup> HSV nucleic acid detected but unable to subtype as HSV-1 or HSV-2

<sup>b</sup> These samples were negative by the artus HSV-1/2 real-time PCR assay; In addition, they were negative following repeat testing by the Roche HSV-1/2 analyte specific reagents.