Performance Comparison and Workflow Evaluation of Simplexa™ HSV 1 & 2 Direct and ARIES® HSV 1 & 2 Assay for the Detection of HSV 1 & 2 in Genital and Non-genital Specimens

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Abstract

Background: Rapid detection of Herpes simplex virus types 1 (HSV 1) and 2 (HSV 2) from genital and non-genital cutaneous or mucocutaneous lesions is of clinical importance for appropriate and prompt treatment of suspected Herpes infections. In this study we compare assay performance and workflow of two molecular assays for the rapid detection of HSV 1 & 2.

Methods: Sixty-six archived specimens (15 genital and 51 non-genital) were simultaneously tested by Simplexa™ HSV 1 & 2 Direct (Focus Diagnostics) and ARIES® HSV 1 & 2 Assay (Luminex Corp.). Specimens were previously identified as HSV 1, HSV 2, or negative by culture or DFA and HSV 1 & 2 monoclonal-antibody staining. Discordant results were repeated once and correlated to clinical presentation. Reproducibility studies were performed using Zephyromics NaThal HSV 1 & 2 controls at low, medium and high concentrations.

Results: Agreement between ARIES, Simplexa and culture or DFA was 100% (66/66) after repeat testing of discordant results and/or clinical correlation. Initial testing on ARIES revealed 1 invalid, 1 false negative (FN) (HSV 1), and 2 false positive (FP) results (one HSV 1 and one HSV 2). Simplexa reported 3 FP results (two HSV 1 and one HSV 2). Upon repeat testing, the ARIES results showed resolution of the invalid as negative (correct) and correct determinations for both the FP and FN HSV 1 results. Similarly, with Simplexa, repeat testing yielded a correct determination for the two FP HSV 1 results. The one culture-negative specimen was positive for HSV 2 by both instrument platforms initially, repeated positive for HSV 2 with both ARIES and Simplexa and was determined to be a true Herpes infection by clinical presentation and serology. Reproducibility studies for HSV 1 (low, medium, high) on ARIES and Simplexa was 100% and 96%, respectively. Reproducibility for HSV 2 (low, medium, high) on ARIES and Simplexa was 93% and 93%, respectively. Evaluation of workflow for pre-analytical hands on time was similar, whereas specimen volume, assay runtime and overall ease of use were dissimilar.

Conclusions: Performance was equal between the Simplexa HSV 1 & 2 Direct and ARIES HSV 1 & 2 Assay. Eight differences were observed for workflow evaluation and overall user preference. Both assays are rapid and performed directly from specimen with increased sensitivity over culture, allowing streamlined options for laboratories and critical benefits in decreased turnaround time and increased clinical sensitivity.

Methods

- 66 archived clinical specimens (15 genital and 51 non-genital) collected between 2/2014 and 4/2015
- Previously identified as HSV 1, HSV 2, or negative by culture and/or DFA, followed by HSV 1 & 2 monoclonal-antibody staining

Method Comparison

- Simultaneously tested by Simplexa™ HSV 1 & 2 Direct (Focus Diagnostics) and ARIES® HSV 1 & 2 Assay (Luminex Corp.)
- Compared to viral culture growth in MRICS and AS49 cells or DFA both stained with Light Diagnostics™ SimFluor HSV 1/2 Immunofluorescence Assay

Discordant Resolution

- Discordant results were repeated once and correlated to clinical presentation

Workflow Evaluation

- Pre-analytical procedure
- Test procedure (Specimen volume, Set up time, Run time)
- Post-analytical procedure
- Overall software ease of use
- Scale: 1= very difficult, 2= difficult, 3= easy, 4= very easy

Results

Method Comparison and Workflow Evaluation

<table>
<thead>
<tr>
<th>Simplexa</th>
<th>ARIES</th>
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<tbody>
<tr>
<td>95.4% agreement with culture/DFA</td>
<td>95.5% agreement with culture/DFA</td>
</tr>
<tr>
<td>1 Invalid</td>
<td>3 False Positives (2-HSV 1, 1-HSV 2)</td>
</tr>
<tr>
<td>1 False Negative (HSV 1)</td>
<td>2 Corrected on repeat (HSV 1)</td>
</tr>
<tr>
<td>2 False Positive (HSV 1, HSV 2)</td>
<td>1 Corrected upon clinical review (HSV 2)</td>
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Time Comparison of Molecular Assay to Viral Culture

<table>
<thead>
<tr>
<th>Simplexa</th>
<th>ARIES</th>
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<tbody>
<tr>
<td>16</td>
<td>120</td>
</tr>
<tr>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>0</td>
<td>128</td>
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</tbody>
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Conclusions

- 95% Agreement with viral culture/ DFA for both ARIES and Simplexa
- All corrected upon repeat or clinical correlation

98% Agreement with viral culture/ DFA for non-genital specimens

Comparable in pre-analytical, analytical and post-analytical test procedure complexity and hands-on time