Validation of Aries HSV 1 and 2 Using Formalin-Fixed Paraffin-Embedded Tissue

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Background
Herpes Simplex Virus (HSV) 1 and 2 are pathogenic viruses of the Herpesviridae family that globally affect approximately 67% of the population younger than 50 and 11% of the population aged 15-49. Traditionally considered to affect oral or genital areas of the body, HSV 1 and 2 can also involve internal structures, such as the esophagus and colon, which are very often diagnosed only by biopsies. Determining the presence or absence of HSV 1 or 2 in formalin-fixed paraffin-embedded (FFPE) biopsy specimens via real-time polymerase chain reaction can be a lengthy procedure that requires up to 16 hours, including approximately 1 hour of hands-on technologist time. As with any manual process, errors can occur, hence the need for a more automated protocol. The Aries HSV 1 and 2 Assay (Luminex, Austin, TX) has not been validated for use on FFPE samples, and this study was undertaken to explore if efficiencies could be gained with this assay.

Methods
Forty retrospective FFPE tissue samples from colon, esophagus, brain, genital, and skin biopsies that had previously been tested for HSV 1 and 2 using the Roche HSV 1 and 2 assay. Negative, HSV 1 positive, and HSV 2 positive FFPE samples were subject to tissue lysis buffer, proteinase-k, and heat (56°C) until completely lysed per the Qiagen DNA Mini kit protocol (Qiagen, Valencia, CA). The lysed cells were then placed in a Qiagen QIAamp spin column and centrifuged for 20s at 8000 RPM to reduce the presence of excess cellular debris. The lysed product was then placed into an Aries HSV 1 and 2 cartridge, and testing was performed on the Aries instrument according to the standard protocol.

Results
The results of the Aries HSV 1 and 2 Assay showed 95% concordance with the current validated HSV 1 and 2 assay. The time required to lyse the FFPE biopsies was the same as previously described, but the hands-on extraction time was lessened by approximately 30 minutes, mitigating errors.

<table>
<thead>
<tr>
<th>Luminex</th>
<th>Aries</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Predictive Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>18</td>
<td>0</td>
<td>18</td>
<td>PPV: 100.00%</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>20</td>
<td>22</td>
<td>NPV: 90.91%</td>
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</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>20</td>
<td>40</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity: 90.00%
Specificity: 100.00%

herent values could be gained with this assay

Discussion
As seen in the H&E stains above, the sample heterogeneity in FFPE samples, as well as smaller tissue sections available, are most likely contributing to the two discordant samples.

Conclusion
The Aries HSV 1 and 2 Assay is an efficient and robust assay that reduces hands-on time in performing HSV 1 and 2 testing of DNA from FFPE, and provides accurate results with a slightly faster turn-around time of results for clinicians and patients.