**Background**

The ARIES® Group A Strep Assay (ARIES® GAS ) in development is a real-time polymerase chain reaction (PCR) based qualitative test for the direct detection of *Streptococcus pyogenes* (GAS) in throat swab samples. The ARIES® System is a fully automated sample to answer system that performs automated nucleic acid extraction, real-time PCR detection of nucleic acid sequences and data analysis. The total ARIES® Group A Strep Assay time from sample to answer is approximately 2 hours.

The objective of this study was to evaluate the performance of the ARIES® Group A Strep Assay in development with throat swab samples.

**Materials and Methods**

**Samples**

The performance of the ARIES® Group A Strep Assay in development was evaluated with 220 remnant throat swab samples. The samples were collected with the COPAN ESwab™ Liquid Amies transport system. The throat swab samples were assessed for the presence of GAS with the Lyra® Direct Strep Assay (Quadel), a real-time PCR based test, and the remnant samples were tested with the ARIES® Group A Strep Assay. Molecular testing was performed within 72 hours of sample collection. Samples were stored at 4°C between collection and testing.

**Bi-directional Sequencing Analysis**

PCR was performed using the HotStarTaq Plus (Qiagen). In brief, 5 μL of nucleic acid extract was used for PCR with M13 tagged analyte specific primers that targeted a genomic region independent from the ARIES® Group A Strep assay. Dye-labeled terminator cycle sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (ThermoFisher) according to the manufacturer’s instructions. Sample electrophoresis and sequence analysis were performed on the 3730xl Analyzer (ThermoFisher) using the 3730xl Data Collection software (v 3.1.1) and Sequencing Analysis software (v 5.4).

**Results**

- A total of 220 remnant throat swab samples were tested with the ARIES® Group A Strep Assay.
- Samples were assessed for the presence of GAS with the Lyra® Direct Strep Assay.
- Discrepant results from the ARIES® GAS Assay compared to the Lyra® Direct Strep assay were subject to bi-directional sequencing for resolution.

**Discussion**

- All 220 samples tested with the ARIES® GAS Assay returned valid results. The internal sample processing control was detected within the assay parameters in each test processed.
- 2 discordant samples were identified as positive by the Lyra® Direct Strep assay and negative by the ARIES® GAS Assay in this study. Both samples were confirmed negative with bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the ARIES® GAS Assay.
- 1 discordant sample was identified as negative by the Lyra® Direct Strep assay and positive by the ARIES® GAS Assay in development with a Ct value of 39.8. This sample tested negative using bi-directional sequencing.
- In this evaluation, the ARIES® GAS Assay detected GAS from remnant throat swab samples with a positive agreement of 97.4% and a negative agreement of 99.3%. Post discrepant resolution, ARIES® GAS Assay detected GAS with a positive agreement of 100%.

**Conclusion**

This study demonstrated that the ARIES® Group A Strep Assay in development offers a simple and rapid sample-to-answer solution for GAS testing. The clinical performance of the assay requires a multi-site clinical study.

**TABLE 1. ARIES® GAS Assay Results Compared with Lyra® Direct Strep**

<table>
<thead>
<tr>
<th>ARIES® GAS Assay</th>
<th>Lyra® Direct Strep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POS</td>
</tr>
<tr>
<td>POS</td>
<td>76</td>
</tr>
<tr>
<td>NEG</td>
<td>2**</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
</tr>
</tbody>
</table>

*1 sample was confirmed negative with bi-directional sequencing

**TABLE 2. ARIES® GAS Assay Percent Agreement with Lyra® Direct Strep**

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>95% Confidence Interval</th>
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<tbody>
<tr>
<td>Positive Percent Agreement</td>
<td>97.4% (76/78)*</td>
<td>91.0%-99.7%</td>
</tr>
<tr>
<td>Negative Percent Agreement</td>
<td>99.3% (141/142)</td>
<td>96.1%-99.9%</td>
</tr>
</tbody>
</table>

*Confidence intervals were calculated with the web calculator http://graphpad.com/quickcalc/ConfInterval1.cfm

*Positive Agreement with results from discrepant resolution is 100% (78/78)