Materials and Methods

Contrived samples were created by suspending American Type Culture Collection (ATCC®) and Zeptometrics® control strains in RPR® MicroTest™ 5® media. Fifty microliters of contrived sample was then added to 1 mL of Bovine Liver Extract to prepare a moderately positive sample for extraction.

Testing was performed using the NxTAG Respiratory Pathogen Panel (RUO) assay kits per the manufacturer’s guidelines. Briefly, 10 μL of internal control was combined with 200 μL of contrived sample prior to extraction. Automated extraction was performed on the Hamilton® Microlab STAR™ (Figure 1) using Promega® Maxwell® HT Viral Total Nucleic Acid kit reagents and custom-designed protocol. Manual extraction was performed using Biomerieux® NucliSENS easyMag® robot, reagents and Generon® 2.01 protocol. For both automated and manual extractions, 35 μL of extracted product was added to the RPP reaction vessel, sealed and placed on a thermal cycler using the RT-PCR protocol described in the package insert (Figure 2). Data was acquired using a MAGPIX® instrument and analyzed with the RPP-assay specific Software Accessory Package using SYNCT software.

Performance was evaluated by extracting a total of 6 replicates of each contrived sample across five Microlab STAR runs. Each set of extracted samples was then tested using NxTAG Respiratory Pathogen Panel (RUO) kit as described above. The same samples extracted on the easyMag instrument and tested using NxTAG RPP (RUO), assay (described above) were used as the comparator method.

Hands-on time (HOT) and automated time (AT) were measured for the following steps using a single Microlab STAR or easyMag: instrument preparation, addition of internal control to extraction well, transfer of sample aliquot from original collection device to extraction well, total nucleic acid extraction, storage of extracted nucleic acid, transfer of extracted product to NxTAG Respiratory Pathogen Panel reaction vessel, thermal cycling, and data acquisition.

Results

Table 1: Comparison of NxTAG RPP total turnaround time using a single Microlab STAR or easyMag.

<table>
<thead>
<tr>
<th></th>
<th>Microlab STAR</th>
<th>easyMag</th>
<th>Microlab STAR</th>
<th>easyMag</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HOT AT</td>
<td>HOT AT</td>
<td>HOT AT</td>
<td>HOT AT</td>
</tr>
<tr>
<td>24 SAMPLES</td>
<td>4 hours 18.6 min</td>
<td>4 hours 56.4 min</td>
<td>4 hours 18.6 min</td>
<td>4 hours 56.4 min</td>
</tr>
<tr>
<td>48 SAMPLES</td>
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<td>6 hours 2.4 min</td>
<td>7 hours 53.4 min</td>
<td>9 hours 46.2 min</td>
</tr>
<tr>
<td>72 SAMPLES</td>
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<td>4 hours 56.4 min</td>
<td>4 hours 18.6 min</td>
<td>4 hours 56.4 min</td>
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<tr>
<td>96 SAMPLES</td>
<td>4 hours 18.6 min</td>
<td>4 hours 56.4 min</td>
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Discussion

Microlab STAR and easyMag workflows were evaluated for 24, 48, 72 and 96 sample batches. The Microlab STAR reduced hands-on time by 30.22%, 68.08%, 109.95%, and 141.81 minutes, respectively.

• Total assay time was slightly higher for the Microlab STAR when running 24 samples compared to the easyMag (Table 1), actual hands-on time by the user was reduced by 31.22 minutes.
• Total assay time and hands-on time were reduced when comparing 48, 72 and 96 sample runs on the Microlab STAR to a single easyMag (Table 2).
• Samples extracted by the Microlab STAR resulted in the correct qualitative results for all replicates across multiple NxTAG RPP (RUO) assay runs (Table 2).

Conclusion

Replacing a traditional total nucleic acid extraction method with the Microlab STAR automated extraction solution reduces hands-on time required to extract samples for the NxTAG Respiratory Pathogen Panel (RUO) assay. The Microlab STAR provides a considerable reduction in total NxTAG Respiratory Pathogen Panel (RUO) assay time when >24 samples are run simultaneously and only a single easyMag instrument is available. No changes to the NxTAG Respiratory Pathogen Panel (RUO) assay’s accuracy and precision were found when comparing the Microlab STAR-extracted samples to traditional methods. Running multiple easyMag instruments simultaneously can reduce the overall assay time for NxTAG Respiratory Pathogen Panel (RUO) assay (data not shown). Due to the easyMag’s increased hands-on time, the Microlab STAR provides a shorter overall assay turnaround time when comparing runs greater than 24 samples. The Microlab STAR’s sample barcode-scanning, digital sample tracking, internal control liquid handling and direct pipetting from the original sample container reduces user time and eliminates points of user interaction that can lead to incorrect result generation and reporting.

Further studies will be required to establish additional performance characteristics of the NxTAG Respiratory Pathogen Panel (RUO) assay with the Microlab STAR automated extraction system. Limit of detection (LOD) was examined. All tests performed, extracted material with the Microlab STAR will be conducted after publication of the easyMag LOD studies.

Acknowledgements

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