Diahrrhea illness represents a large public health burden with a high degree of morbidity and mortality among children, the elderly, and immunocompromised. The WHO estimates that annually there are two billion cases of diarrheal disease worldwide.1 Accurate and rapid testing is essential for the treatment and management of diarrheal disease. The Prodiagnost STEC (PGCd) and the ProGastro™ SSICS (PGGSSICS) Assays are real-time PCR assays used to detect toxicogenic Clostridium difficile (cDc) and Salmonella. Shigella, Campylobacter (c.junii, c.coli only, not differentiated), and Shiga Toxin producing E. coli (STEc, stx1 and stx2 differentiated, respectively). For the package inserts, both assays utilize the BioMérieux NuSiSENS easyMAG for extraction; however, they incorporate unique sample processing and easyMAG protocols. A panel of stool samples was processed via both assay’s extraction methods and then tested with both assays. This allowed for the assessment of each processing method when used in conjunction with the PGCd and PGGSSICS Assays. The panel consisted of eight contigent samples for each target detection and was generated from individual negative raw stool samples along with 40 individual negative samples. A portion of each sample was prepared and extracted via the package insert for the PGCd and PGGSSICS Assays. Purified nucleic acids were tested immediately after extraction with the real-time PCR mix from both assays. The remaining nucleic acids were then stored frozen, thawed, and tested again using both assays to assess any effects due to freezing. The results of the testing via the two different preparation methods were compared and statistically analyzed.

For both the fresh and frozen nucleic acids, there was a statistical difference (p<0.05) between the two methods for the Salmonella, Shigella, and STEc method resulting in better sensitivity. There was no statistical (p>0.05) difference between the two methods for the Campylobacter and stx2 detections. Therefore, there is a need for testing one sample for the targets addressed by both the PGCd and PGGSSICS Assays, processing samples according to the method of the PGGSSICS Assay allows for a single nucleic acid sample to be used in conjunction with both assays with acceptable performance.

Due to the large burden of diarrheal illness, it may be advantageous to be able to process/extract a single sample for use with both the PGCd and PGGSSICS Assays. Therefore, a panel of continuous specimens with target organisms at various concentrations based on their particular Limit of Detection (LoD) was generated and processed/extracted via each assay’s package insert utilizing the bioMérieux NuSiSENS easyMAG. This allowed for a comparison between processing methods when the nucleic acids generated from each method were tested with both the PGCd and PGGSSICS assay’s real-time PCR mixes.

## Methods

### Conting Sample Panel

Generated from 80 leftover individual PGCd and PGGSSICS negative raw (liquid/soft) stool samples (two each at 2x, 5x, 10x, and 100x LoD) for the Salmonella, Shigella, Campylobacter, STEC, and stx2 groups. For the stx1 and stx2 groups, the dilutions were 2x at 2x, 5x, 10x, and 100x LoD.

## Results Cont.

### Variability Charts Comparing the Two Processing/Extraction Methods (Examples)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Mean Difference - Fresh vs. Frozen by Method</th>
<th>Statistical Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGCd</td>
<td>Mean difference for each sample type is calculated as follows: Mean difference = Mean鲜鲜 - Mean鲜冻</td>
<td>Fresh vs. Frozen by Method</td>
</tr>
<tr>
<td>PGGSSICS</td>
<td>Mean difference for each sample type is calculated as follows: Mean差差 - Mean差鲜</td>
<td>Fresh vs. Frozen by Method</td>
</tr>
</tbody>
</table>

## Conclusions

Based on the comparison data and the fact that the STAR method was unable to detect agents of gastrointestinal illness, the STAR method was the only method tested and was the only sample for the targets addressed by both the PGCd and PGGSSICS Assays, processing samples according to the method of the PGGSSICS Assay (CAS method) allows for a single nucleic acid sample to be used in conjunction with both assays with acceptable performance.

### NOTE: Testing for Clostridium difficile, it is recommended to add the raw stool sample to the CAS just prior to processing/extraction. C. difficile is an anaerobic organism and it has been observed in our lab that once the raw stool is placed in CAS, its detection decreases by ~ 1 log in the 1st 24 hours and then is unable to be detected by 48 hours post addition to CAS.

Gastroenteritis affects a large portion of the population every year. Real time PCR assays allow for the detection of agents of gastrointestinal illnesses in hours rather than days as with culture and provide a means for more efficient patient treatment and management.

## References