The ProGastro SSCS assay was able to detect bacterial pathogens (Salmonella, Shigella, and STEC) in 16 of 122 (13%) specimens tested.

In contrast, the conventional microbiological tests only detected a pathogen in 6.5% (8) tested.

As shown in Table 1 the ProGastro SSCS Assay was able to detect additional 6 Salmonella, 1 Shigella and 1 STEC positive sample(s) that were undetected by conventional microbiological methods and these were confirmed by sequencing.

No Campylobacter was detected by culture or the ProGastro methods.

### Abstract

**Background:** Gastrointestinal infections are a major health problem causing significant morbidity and mortality worldwide. Conventional microbiological methods can be complex and time consuming, leading to misidentification and lengthy turnaround times. The ability to detect gastrointestinal pathogens using molecular methods from stool samples may significantly increase the accuracy and timeliness of results. The objective of this study was to evaluate the performance characteristics of the Prodesse ProGastro™ SSCS Assay (Biotic Gen-Probe, Inc., CA) as compared to conventional methods. This is an in vitro qualitative multiplex Real-Time PCR assay for the qualitative detection and differentiation of Salmonella, Shigella, and Campylobacter (C. jejuni and C. coli only, undifferentiated) nucleic acids and Shiga Toxin 1 (stx1) and Shiga Toxin 2 (stx2) genes.

**Methods:** Evaluation of the assay was performed on preserved stool samples from symptomatic pediatric and adult patients admitted for routine testing to Texas Children's Hospital from September to December 2011. All samples were subjected to the current, approved in-house culture methods for Salmonella, Shigella and Campylobacter. Enzyme immunoassay (Meridian Premier HEIEC) for STEC was performed in accordance with the manufacturer's instructions. For the Prodesse ProGastro SSCS assay, stool samples were placed in stool preservative and transport media and processed to isolate nucleic acids using the Nuclisens easyMAg automated extraction system (BioMerieux, NC). Multiplex PCRs were performed according to the manufacturer's instructions using the ProGastro SSS Investigational Use Only (IUO) reagents (at the time of testing) on the Cepheid SmartCycler II instrument. Discrepant results between the methodologies were resolved by bi-directional sequencing.

**Results:** During the study period, the assay was evaluated by testing 122 patient stool samples presenting to Texas Children's Hospital with clinical features suggestive of a gastrointestinal infection. Of these, the ProGastro SSCS Assay detected pathogens (Salmonella, Shigella and STEC) in 13% (16) of the samples. In contrast, the conventional microbiological tests only detected a pathogen in 6.5% (8) tested. Furthermore, the ProGastro SSCS Assay was able to detect additional 6 Salmonella, 1 Shigella and 1 STEC positive sample(s) that were undetected by conventional microbiological methods and these were confirmed by sequencing. No Campylobacter was detected by either method.

**Conclusions:** The ProGastro SSCS Assay is a powerful tool in the detection of gastrointestinal pathogens and showed excellent sensitivity and specificity across the full range of pathogens it has been designed to detect, and has the ability to detect, when measured against conventional microbiologic testing. Additionally, this assay has the potential to provide rapid and accurate diagnosis of gastrointestinal infections by increasing the diagnostic yield and shortening the turnaround time (in under 4 hours) when compared to days or weeks with the current standard of care.

### Methods

- **Sample Size:** 122 clinical specimens
- **Inclusion criteria:** All preserved stool samples from symptomatic pediatric and adult patients admitted for routine testing to Texas Children's Hospital from September to December 2011
- **Exclusion criteria:** Repeat isolates
- **Bacterial culture:** Bacterial isolation from stool culture is the gold standard for detection of gastrointestinal pathogens and the reference method against which all other tests are measured. All samples were subjected to the current, approved in-house culture methods for Salmonella, Shigella and Campylobacter species. Briefly, all stool samples were inoculated onto 5% Sheep Blood agar, MacConkey agar, Xylose Lysine Desoxycholate agar(XLD), and Campylobacter media (Campy Thio and Cefoperazone/Vancocmycin,Amphothericin B with 5% Sheep blood agar (CSA)), incubated at the appropriate atmosphere; and examined for growth of Campylobacter, Salmonella, Shigella, and Verocya by standard methods. E. coli O157 was detected by culturing on Chromagar O157 and sorbitol-MacConkey agar.
- **Meridian Premier HEIEC Assay:** Enzyme immunoassay (EIA) for Shiga toxin 1 and 2 using the Meridian's Premier assay was performed in accordance with the manufacturer's instructions. The Premier HEIEC assay employs monoclonal anti-Shiga toxin 1 and 2 antibodies to capture the toxin and horseradish peroxidase-labeled polyclonal anti-Shiga toxin 1 and 2 to detect the bound toxin. Briefly, the stools were enriched in MacConkey broth at 37°C for 16 to 24 h before testing. The broth supernatant was then added to the antibody coated wells and EIA testing was performed.
- **Prodesse ProGastro™ SSCS Assay:** All stool samples were placed into Cary-Blair preservation and transport media. A 100 μL of the diluted stool specimen was processed to isolate nucleic acids using the Nuclisens easyMAg automated extraction system (BioMerieux, NC). A 14 μL of the prepared Gastro RNA/DNA Internal Control (GIC, Gen-Probe Prodesse, Wausauka, USA) was added to each specimen before extraction. Multiplex PCRs were performed according to the manufacturer's instructions using the ProGastro SSS Investigational Use Only (IUO) reagents (at the time of testing) on the Cepheid SmartCycler II instrument (Cepheid, CA, USA). A 25 μL PCR reaction consisted of 5 μL nucleic acid of the samples or positive controls (Salmonella, Shigella, C. jejuni, C. coli and Shiga toxin producing E. coli) and 20 μL of SSC or STEC mix, respectively. The negative control consisted of 5 μL molecular grade water and 20 μL SCC or STEC mix, respectively.
- **Discrepant results between the methodologies were resolved by bi-directional sequencing.

### Results

<table>
<thead>
<tr>
<th>Prodesse ProGastro™ SSCS Assay</th>
<th>Results of Conventional Microbiological testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>13</td>
</tr>
<tr>
<td>Shigella</td>
<td>1</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>0</td>
</tr>
<tr>
<td>stx1/stx2</td>
<td>2 for stx1</td>
</tr>
</tbody>
</table>

**Table 1. Performance of the ProGastro SSCS assay in Comparison to Conventional methods**

### Conclusions

- The ProGastro SSCS Assay detected bacterial pathogens in 13% of the specimens tested in contrast to the 6.5% that were detected by the conventional microbiological methods.
- The ProGastro assay is a powerful tool for providing rapid diagnosis of gastrointestinal infections by increasing the diagnostic yield and decreasing the turnaround time (in under 4 hours) when compared to days or weeks with the current standard of care.

### Acknowledgments

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