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Application of Alternative Nucleic Acid Extraction Protocols to ProGastro SSCS Assay for Detection of Bacterial Enteric Pathogens

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As an alternative to automated extraction, fecal specimens were processed by investigational lysis/heating (i.e., manual) and by chromatography/centrifugation (i.e., column) methods. ProGastro SSC and Shiga toxin-producing Escherichia coli (i.e., STEC) indeterminate rates for 101 specimens were 1.0% to 3.0% for automated, 11.9% for manual, and 24.8% to 37.6% for column methods. Following freeze-thaw of 247 specimens, indeterminate rates were 1.6% to 2.4% for manual and 0.8 to 5.3% for column methods. Mean processing times for manual and column methods were 30.5 and 69.2 min, respectively. Concordance of investigational methods with automated extraction was ≥98.8%.

Accurate and rapid laboratory diagnostics for patients with gastroenteritis contribute to clinical management and expedient public health notification (1). Molecular-based assays have demonstrated reliable identification of common bacterial agents of gastrointestinal disease (2–6), yet performance of these assays may present obstacles for laboratories not possessing automated means of nucleic acid extraction and workflow challenges for other laboratories. Development of alternative processing protocols must ensure mitigation of inhibitory agents endogenous to fecal specimens (7, 8) and of those produced by commensal enteric Gram-negative bacteria (9).

ProGastro SSCS (Hologic, San Diego, CA) is an FDA-cleared multiplex real-time PCR assay for qualitative detection of nucleic acid specific to Salmonella spp., Shigella spp., Campylobacter coli/ Campylobacter jejuni (undifferentiated), and Shiga toxin-producing Escherichia coli (STEC). The assay is indicated on preserved fecal specimens from symptomatic patients with signs and symptoms of gastrointestinal. Furthermore, performance of PCR is to be preceded by nucleic acid extraction using either of two automated platforms (10). Compared to conventional culture/enzyme immunoassay modalities, and paired with a bidirectional sequencing adjudicator, the assay demonstrated 100% accuracy in detection of these nucleic acids from preserved fecal specimens (6). Valid results were procured at a rate of 98.8% using NucliSens easyMag system (bioMérieux) automated extraction.

One commercial molecular assay for another gastrointestinal pathogen, Clostridium difficile, utilizes facets of manual extraction paradigms described by Boom et al. (11). Importantly, glass particles were found to bind nucleic acids in the context of crude cellular lysates. Previous data describe efficient manual processing protocols for molecular detection of toxigenic C. difficile with rapid turnaround time (12). Moreover, quality assurance data revealed a 0.51% molecular C. difficile indeterminate rate (indicative of insufficient internal control amplification) from 10,131 primary specimens tested over 3 years (Wheaton Franciscan Laboratory, unpublished findings). The objective of this study was to investigate application of off-label, nonautomated extraction protocols, including methods previously described by Boom et al. (11), to clinical performance of ProGastro SSCS. (Results of this work were presented in part at the 115th General Meeting of the American Society for Microbiology, New Orleans, LA, 30 May to 2 June 2015.)
lar lysates (pulse-centrifuged for 10 s) were incubated in both STEC (detection of internal control sequence and nucleic acids specific to E. coli Shiga toxin) and SSC (detection of internal control sequence plus nucleic acids specific to Salmonella spp., Shigella spp., and C. coli/C. jejuni) master mixes as components of ProGastro SSCS (10). Per package insert specifications, the maximum cycle threshold (CT) value yielding a valid internal control nucleic acid amplification result on both SSC and STEC master mixes was 45.0.

Efficacy of extraction modalities for amplification of internal control was assessed in the context of 101 clinical specimens (referred to as Cary Blair stool) and 16 Cary Blair aliquots. Of the 702 total results generated by the three extraction modalities distributed among SSC and STEC master mixes, 124 (17.7%) were initially discordant, with 117 of these being indeterminate (Table 1). An unacceptable percentage of indeterminate SSC results was generated following manual (11.9%) and column (24.8%) extractions of Cary Blair stool ($P_{/H11349}0.016$ versus automated). When the mean CT values of specimens that did yield valid results for all three extraction modalities were compared, less internal control amplification was generated by column ($P_{/H11021}0.0001$ versus manual) and manual ($P_{/H11021}0.0001$ versus automated) methods. Moreover, 75% of Cary Blair aliquots failed to generate a valid result via column extraction, with decreased internal control amplification ($CT$, 40.05; $P_{/H11021}0.013$ versus automated and manual). Similar findings resulted from incubation of automated, manual, and column extracts/lysates with the STEC master mix.

As studies have espoused the value of a freeze-thaw cycle for removal of endogenous inhibitors from primary specimens (14–16), extractions were subsequently processed following storage of 247 Cary Blair stools and 39 Cary Blair aliquots overnight at $−70^\circ\text{C}$. The 101 previously assessed Cary Blair stools (Table 1) were a component of this study set. Of the 1,716 total results generated by the three extraction modalities distributed among SSC and STEC master mixes, just 58 (3.4%) were initially discordant, with 47 of these being indeterminate (Table 2). Valid Cary Blair stool results were generated from SSC master mix at a rate of $\geq98.4\%$ after automated, manual, and column extraction ($P_{/H11021}0.41$). Both automated and manual extraction allowed for sufficient internal control amplification from Cary Blair stool and Cary Blair aliquots ($CT_{/H11021}34.82; P_{/H11021}0.22$ for intermodality comparisons). An unacceptable indeterminate result rate from Cary Blair aliquots remained for column extraction (20.5%), while internal control amplification was less efficient ($CT$, 39.67; $P_{/H11021}0.0001$ versus automated and manual). Also, 5.3% and 28.2% of Cary Blair stool and Cary Blair aliquot STEC master mix results, respectively, derived from column extraction were indeterminate. Internal control amplification was less robust than automated and manual extraction ($P_{/H11021}0.0001$). Manual extraction of clinical and control material exhibited similar performance to automated ex-

### TABLE 1 Characterization of initial ProGastro SSCS results derived from three extraction modalities on 101 Cary Blair-preserved stool specimens and 16 Cary Blair aliquots without antecedent freeze-thaw cycle

<table>
<thead>
<tr>
<th></th>
<th>SSC master mix</th>
<th></th>
<th>STEC master mix</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cary Blair stool</td>
<td>Mean internal control $CT^{a}$</td>
<td>No. (%) of indeterminate results</td>
<td>Mean internal control $CT^{a}$</td>
</tr>
<tr>
<td>Automated</td>
<td>3 (3.0)</td>
<td>34.95</td>
<td>0 (0.0)</td>
<td>35.43</td>
</tr>
<tr>
<td>Manual</td>
<td>12 (11.9)</td>
<td>36.55</td>
<td>0 (0.0)</td>
<td>35.60</td>
</tr>
<tr>
<td>Column</td>
<td>25 (24.8)</td>
<td>38.42</td>
<td>12 (75.0)</td>
<td>40.05</td>
</tr>
</tbody>
</table>

$^{a}$ Provided for specimens yielding detectable internal control amplification with all three extraction modalities.

$^{b}$ Differences between automated, manual, and column ($P_{/H11021}0.54$).
Characterization of initial ProGastro SSCS results derived from three extraction modalities on 247 Cary Blair-preserved stool specimens and 39 Cary Blair aliquots following a single freeze-thaw cycle

<table>
<thead>
<tr>
<th>Extraction modality</th>
<th>Cary Blair stool</th>
<th>Cary Blair aliquots (negative control)</th>
<th>STEC master mix</th>
<th>Cary Blair stool</th>
<th>Cary Blair aliquots (negative control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%) of indeterminate results</td>
<td>Mean internal control C₀ indications</td>
<td>No. (%) of indeterminate results</td>
<td>Mean internal control C₀ indications</td>
<td>No. (%) of indeterminate results</td>
</tr>
<tr>
<td>Automated</td>
<td>2 (0.8)</td>
<td>34.57</td>
<td>0 (0.0)</td>
<td>34.82</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Manual</td>
<td>4 (1.6)</td>
<td>34.34</td>
<td>0 (0.0)</td>
<td>34.81</td>
<td>6 (2.4)</td>
</tr>
<tr>
<td>Column</td>
<td>2 (0.8)</td>
<td>36.58</td>
<td>8 (20.5)</td>
<td>39.67</td>
<td>13 (5.3)</td>
</tr>
</tbody>
</table>

*Provided for specimens yielding detectable internal control amplification with all three extraction modalities.

Nucleic acid detection specific to:

- **Salmonella spp.**
- **Shigella spp.**
- **Campylobacter coli**
- **Campylobacter jejuni**
- **Shiga toxin**

**Sensitivity (%)**

<table>
<thead>
<tr>
<th>Performance index by extraction modality</th>
<th>Nucleic acid detection specific to:</th>
<th>Automated</th>
<th>Manual</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella spp.</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Shigella spp.</td>
<td>100.0</td>
<td>91.7</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>Campylobacter coli/Campylobacter jejuni</td>
<td>100.0</td>
<td>100.0</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>Shiga toxin</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Specificity (%)**

<table>
<thead>
<tr>
<th>Performance index by extraction modality</th>
<th>Nucleic acid detection specific to:</th>
<th>Automated</th>
<th>Manual</th>
<th>Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella spp.</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Shigella spp.</td>
<td>100.0</td>
<td>99.6</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Campylobacter coli/Campylobacter jejuni</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Shiga toxin</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

determine if rates of initially indeterminate results can be decreased to the level of the manual method. In our evaluation, the initial dilution was accomplished with consecutive 1:2 and 1:5 dilutions to allow for a larger sampling of Cary Bair stool. Despite the increased labor, mean processing time for manual extraction of a 7-specimen batch (30.5 min) was less than both the column method (69.2 min; P < 0.0001) and the automated reference (60 min; independent laboratory, personal communication). These data do not consider the time necessary to execute the freeze-thaw cycle itself. However, these components can easily be managed by laboratory support personnel as the preserved specimen arrives in the laboratory and by a technologist as part of the routine testing workload.

Similar to other PCR (12, 18, 19) and reverse transcriptase PCR (14) assays, additional workflow optimization of ProGastro SSCS can be accomplished by utilization of batch-prepared frozen master mix tubes (20). In summary, exposure of Cary Bair stool and medium aliquots to a single freeze-thaw cycle accommodates off-label nucleic acid extraction for accurate performance of ProGastro SSCS. This modification, particularly in the context of an efficient lysis protocol, may expand opportunities for laboratories to contribute to the rapid diagnosis of bacterial gastroenteritis.

**ACKNOWLEDGMENTS**

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**REFERENCES**


