EasyScreen™ Molecular Diagnostic Assays for Routine Detection of Faecal Pathogens

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## Average Workload per Month

<table>
<thead>
<tr>
<th>Test</th>
<th>Samples per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>540</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>330</td>
</tr>
<tr>
<td><em>Giardia/Cryptosporidium EIA</em></td>
<td>140</td>
</tr>
<tr>
<td>Rotavirus/Adenovirus EIA</td>
<td>90</td>
</tr>
<tr>
<td>Norovirus EIA</td>
<td>60</td>
</tr>
<tr>
<td>Ova/Cysts/Parasites</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1230</strong></td>
</tr>
</tbody>
</table>
### Testing algorithm

<table>
<thead>
<tr>
<th>Request Type</th>
<th>Stool Type</th>
<th>Rota Virus</th>
<th>Adeno Virus</th>
<th>Direct</th>
<th>Conc</th>
<th>Culture Bacterial</th>
<th>Vibrio Culture</th>
<th>YSA</th>
<th>AMP</th>
<th>C. diff</th>
<th>F G/C</th>
<th>Microsp</th>
<th>EM</th>
<th>AFB</th>
<th>Harada</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Routine M/C/S</td>
<td>Unformed</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Routine M/C/S Child &lt; 5 yrs May-Jan</td>
<td>Unformed</td>
<td>If +</td>
<td>Pos</td>
<td>-</td>
<td>+</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>* Routine M/C/S Child &lt; 5 yrs Feb-Apr</td>
<td>Unformed</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>* Routine M/C/S Child6-12 yrs</td>
<td>Unformed</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>* HIV Pos OPD-B</td>
<td>Unformed</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Outbreak &lt; 2yrs Related Cases</td>
<td>1</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Overseas Traveller OPD-G</td>
<td>Formed</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>* Other Immunocompromised Patients M/C/S</td>
<td>Unformed</td>
<td>+</td>
<td>1</td>
<td>+</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>* Ova/Cysts/Parasites</td>
<td>Formed or Unformed</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>* C. diff Toxin</td>
<td>Unformed</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>**</td>
</tr>
</tbody>
</table>

* Only a single specimen cultured for acute diarrhoea

** 3 Specimens cultured in 7 days for HIV patients ? Immunocompromised

*** C. difficile – positive stool – No repeat in 7 days

- negative stool – Maximum 1 further repeat examination during following 7 days

1. Done on request
2. Watery specimen
3. Done if no pathogens isolated
4. Done on patients – greater than or equal to 48 hours hospitalisation or antibiotic use
Genetic Signatures Sample Preparation and Detection kits
# Gastrointestinal Screening Panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Panel Member</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasyScreen™ Enteric Bacterial Detection Kit (Cat#EB001)</td>
<td><em>Salmonella</em> spp., <em>Shigella</em> spp., <em>Campylobacter</em> spp., <em>Yersinia entercolitica</em>, <em>Listeria monocytogenes</em>, <em>C. difficile</em>, Extraction control and Internal Process Control</td>
</tr>
<tr>
<td>EasyScreen™ Enteric Protozoan Detection Kit (Cat#EP001)</td>
<td><em>Giardia intestinalis</em>, <em>Cryptosporidium</em> spp, <em>Entamoeba complex</em>, <em>Dientamoeba fragilis</em>, <em>Blastocystis hominis</em>, Extraction Control and Internal Process Control</td>
</tr>
<tr>
<td>EasyScreen™ Enteric Viral Detection Kit (Cat#EV001)</td>
<td>Norovirus group I, Norovirus group II, Adenovirus hexon, Adenovirus 40/41, Rotavirus A and B, Astrovirus (group 1-7), Sapovirus, Extraction Control in each</td>
</tr>
</tbody>
</table>
Technology

• *EasyScreen™* assays utilize a novel chemistry called 3base™
  – Universally modifies the nucleic acid sequence of pathogens
    • Converts all Cytosine bases to Uracil during 95°C 15 min incubation to create 3base™ DNA and RNA
    – Improves the efficiency of real-time PCR by reducing the temperature variability in multiplexed reactions

• All reagents apart from those required for extraction are provided in the kit (new version provides all reagents)

• Results in 3-4 hours, greatly reducing the time of diagnosis compared to conventional microbiological techniques
EasyScreen™ Sample Processing Workflow & Platform Compatibility

1. Inoculate stool swab into EasyScreen™ reagent
   Heat for 15 mins

2. Extract sample using spin columns or automated platform

3. Centrifuge (Qiagen only)
   Ready for PCR

**Robotic NA Extraction**
- **Qiagen** (M48, QIAsymphony, EZ1)
- Roche MagNApure systems
- Themo KingFisher Flex
- Biomerieux EasyMag
- Genetic Signatures Robot (Hamilton)
- Versant Sample Prep Chemistry
EasyScreen™ Workflow & Platform Compatibility

Mix Master Mix
Aliquot into 96 well plate (Mastermix x7)

Add 2μL eluate (x 7 per patient)

Centrifuge plate
Load thermocycler

Real Time PCR Platform
Roche Lightcycler™ II and 480
ABI Fast7500
Cepheid SmartCycler
Qiagen RotorGene
Biorad CFX96
Stratagene/Agilent Mx
Loading the 96 well plate

Bacterial A
Bacterial B
Parasite A
Parasite B
Viral A
Viral B
Viral C
Loading the 96 well plate

Sample 1
Loading the 96 well plate
Assay Results: Compatible with a number of instruments

Roche LC480

Rotorgene
Assay Results: Compatible with a number of instruments

Bio-Rad

Agilent
## Results (390 samples)

<table>
<thead>
<tr>
<th>Pathogen detected</th>
<th>EasyScreen™</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Additional pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses (Noro, Rota, Adeno, Astro)</td>
<td>69</td>
<td><strong>100</strong></td>
<td>97.1%</td>
<td>25</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>58</td>
<td>84.8</td>
<td>99.4</td>
<td>9</td>
</tr>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>48</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>0</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>42</td>
<td>97.7</td>
<td><strong>100</strong></td>
<td>1</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>11</td>
<td><strong>100</strong></td>
<td>81.8</td>
<td>0</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em></td>
<td>3</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>2</td>
</tr>
<tr>
<td><em>D. fragilis</em></td>
<td>10</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>10</td>
</tr>
<tr>
<td><em>B. hominis</em></td>
<td>17</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>16</td>
</tr>
<tr>
<td><em>G. intestinalis</em></td>
<td>12</td>
<td>92.3</td>
<td><strong>100</strong></td>
<td>7</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>3</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td>3</td>
</tr>
<tr>
<td><em>Entamoeba</em> complex</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>279</td>
<td></td>
<td></td>
<td>79</td>
</tr>
</tbody>
</table>
Sensitivity

- 285 pathogens detected by either method
  - 10 unable to be confirmed
    - 5 - no confirmatory test available
    - 5 - insufficient sample to confirm
- **EasyScreen™ assay** correctly identified **269 (97.8%)**
- EasyScreen™ assay failed to detect 6 positive samples (*Salmonella* (1), *Giardia* (1) and *C. difficile* (4))
- **Routine testing** (based upon request by attending Physician) correctly identified **202 (73.5%)**
- **Additional pathogens** EasyScreen™ positive (79) where sufficient sample remained were further confirmed as positive by routine test, PCR or tissue culture
Specificity

• No cross contamination between samples

• 100% specificity all pathogens except Shigella, Astrovirus and C. difficile
  – Two C. difficile positive samples that were positive by EasyScreen™ were negative by the Xpert® C. difficile test and culture
  – Two discrepant Astrovirus EasyScreen™ positive assay results (positive adjusted to <35 cycles)
  – Two discrepant EasyScreen™ Shigella-positive/culture-negative samples (quantitation: low copy numbers, negative at independent laboratory)
Cost

• Conventional testing (if testing same targets as EasyScreen™)
  ▪ ~$70/sample
  ▪ Labour costs included ~$120

• EasyScreen™ assays (19 targets)
  ▪ ~$60
  ▪ Labour costs variable (sample volume and extraction platform dependent)
Set Up Time savings (average day)

- GS Assay: Average = 3 min/sample
- Automation: Average = 1-2 min/sample
- Conventional: Average = 5-6 min/sample

Graph showing time savings for various tasks compared to conventional and GS methods.
Sample Inhibition

• Initial study 41 (10.5%) samples extraction control and internal control negative

• After 1:5 dilution of eluate, 37 of the samples achieved correct results
  – Re-extraction needed for the remaining 4 samples

• Technique review
  – Minimise sample overload prior to the heat-extraction step
  – Adequate centrifugation of template prior to testing reduced control failures to <1%
Assay Advantages

• Specificity/Sensitivity
  – No cross contamination over 390 samples
  – Additional pathogens
  – Increased sensitivity compared to EIA
  – Multiple infections (39)

• Additional targets included
  – Adenovirus hexon, Astrovirus, Y. enterocolitica, L. monocytogenes

• Flexibility: 7 Mastermix choices
Assay Advantages

• Versatile platform choices for extraction and amplification
• Time to result
Improvements/Alerts

• Automation will reduce
  – Repetitive pipetting
  – Hands-on-time
  – Eliminate set up errors

• 384 well plate for high throughput

• Eluate unable to be used for other assays
  – Usually adequate primary sample
Acknowledgements

• Tom Olma
• John Melki
• Douglas Millar
• Staff on stool processing bench
• Independent confirmatory PCR/OCP/Tissue Culture
  – Diane Grote, Virology Department, The Children's Hospital at Westmead, Westmead
  – Damien Stark, Division of Microbiology, SydPath St. Vincent's Hospital, Darlinghurst
  – Steven Siarakas, Department of Microbiology and Infectious Diseases, Concord Hospital, Hospital Rd, Concord
  – Rogan Lee, Parasitology, CIDMLS, Westmead Hospital, Westmead
  – Ken McPhee, Viral Laboratory, CIDMLS, Westmead Hospital, Westmead
  – Susie Roczo-Farkas, Enteric Virus Group, The Royal Children’s Hospital, Victoria