Validation of a novel test for hypervirulent strains of *Clostridium difficile* including BI/NAP1/027

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Increasing incidence of *Clostridium difficile* infection (CDI) reported

http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1296681242219
Clostridium difficile infection: The most common nosocomial infection

Superbug Clostridium Difficile About to Overtake MRSA in Hospitals

by Robert Rister / Healthy Living

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- Antibiotics for Clostridium difficile Overgrowth: Both Cause and Cure
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"Super-bug" - MRSA or methicillin-resistant Staphylococcus aureus
For over seven years the news has been filled with scary reports about the "super-bug" MRSA, methicillin-resistant Staphylococcus aureus. This flesh-eating bacterium has been known to haunt
Variability in *C. difficile* toxinotypes = difficulties in detection?

Markers of hypervirulent *C. difficile*

- *tcdC* single base pair deletion at nt 117 ($\Delta117tcdC$)
  - found in 027 and 078 ribotypes
- repeat unit deletion downstream of $\Delta117tcdC$
  - 18bp in 027 ribotype
  - 39bp in 078 ribotype
- Threonine to Isoleucine mutation (codon 82) at *DNA gyrase A* gene (*gyrA*)
  - confers resistance to fluoroquinolone in newer hypervirulent strains (027, 078)
- Presence of binary toxin genes (*cdtA* and *cdtB*)
Introducing the EasyScreen *C. difficile* assays

- **Detection assay**
  - *tcdA* and *tcdB*
  - PCR amplification control
  - Extraction control

- **Reflex assay**
  - Identifies hypervirulent strains
    - “Quebec” strain (BI/NAP1/027)
    - *binary toxin* gene (*cdtA*)
    - *tcdC* single base pair deletion at nucleotide 117 (**Δ**117tcdC)
    - fluoroquinolone resistance marker (*gyrA*)
  - PCR amplification control
Rapid and automatable work flow

Add clinical sample (Stool Swab) into HGS reagent. Heat for 15 mins

Purify sample via columns or magnetic beads via automation (includes elution step with HGS buffer)

Ready for PCR

Total Hands-on time ~ 10 mins/ Incubation times ~ 15 mins/ Sample to result time ~ <3 hrs
Instrument compatibility

• Compatible with multiple extraction platforms.
  – QiaSymphony SP
  – Qiagen Biorobot M48
  – Qiagen Biorobot EZ1
  – Roche MagNApure
  – Thermo Fisher Scientific’s Kingfisher

• Compatible with various real-time PCR systems
  – Roche LightCycler 480 instruments
  – Biorad CFX96
  – Agilent (Stratagene) MX3000
  – Qiagen Rotorgene-Q (or Corbett Rotorgene 6000)
  – Cepheid Smartcycler
Sensitive detection of \textit{tcdA} and/or \textit{tcdB}

- \textasciitilde 20 cfu of \textit{tcdA} or \textit{tcdB} positive \textit{C. difficile} in stool
  - Equivalent \textasciitilde 1 cfu/PCR
Specific detection of \textit{tcdA} and/or \textit{tcdB}

- Does not cross react with
  - Closely related \textit{Clostridium sp.}
    - \textit{C.perfringens}
    - \textit{C.sordelli}
  - Common enteric pathogens
    - \textit{Campylobacter jejuni}
    - \textit{Salmonella enterica}
    - \textit{Shigella flexneri}
    - \textit{Escherichia coli}
    - \textit{Listeria monocytogenes}
    - \textit{Bacillus cereus}
  - \textit{Helicobacter pylori}
  - Parasites (data not shown)
    - \textit{Giardia}
    - \textit{Cryptosporidium}
    - \textit{Entameoba}
    - \textit{Dientameoba}
  - \textit{Candida sp.}
  - \textit{Streptococcus sp.}
  - \textit{Staphylococcus sp.}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{cross_reactivity}
\caption{Cross-Reactivity: \textit{TcdA/B}}
\end{figure}

\textasciitilde 220 cfu of 027 \textit{C.difficile}

\textasciitilde 5 cfu of 027 \textit{C.difficile}

Toxigenic \textit{C.difficile} from stool
# EasyScreen kit results

<table>
<thead>
<tr>
<th></th>
<th>Set 1 (n=70)</th>
<th>Set 2 (n=73)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>%</td>
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<tr>
<td>Culture</td>
<td>67/70</td>
<td></td>
</tr>
<tr>
<td>EIA¹</td>
<td>43/70</td>
<td>61.4</td>
</tr>
<tr>
<td>Illumigene²</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>QuikChek³</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td><em>EasyScreen</em> Detection⁴</td>
<td>53/70</td>
<td>75.7</td>
</tr>
<tr>
<td><em>EasyScreen</em> Reflex⁴</td>
<td>12/70</td>
<td>17</td>
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</table>

Screening results for the 143 samples comparing
1. Techlab ® Wampole™ *C. difficile* Tox A/B II™ EIA,
2. Meridian Bioscience illumigene™ *C. difficile* DNA amplification assay,
3. TechLab® Quik Chek Complete™ and
4. Human Genetic Signatures EasyScreen™ *C. difficile* methods.
# Ribotyping results

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample ID</th>
<th>Binary toxin</th>
<th>Δ117 tcdC</th>
<th>gyrA</th>
<th>tcdC repeat deletion</th>
<th>027 Ribotype</th>
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<tr>
<td>1</td>
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<td></td>
</tr>
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<td>2</td>
<td>Reference 027</td>
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<td>3</td>
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<td>38bp</td>
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<td>Water control</td>
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</table>
### Interpretation of results

<table>
<thead>
<tr>
<th>TcdA/B</th>
<th>Δ117 tcdC</th>
<th>Binary toxin</th>
<th>gyrA</th>
<th>IPC</th>
<th>Status</th>
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<td>- / +</td>
<td>- / +</td>
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<td>Negative</td>
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<td>+</td>
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<td>Toxigenic <em>C. difficile</em></td>
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<tr>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>Toxigenic <em>C. difficile</em></td>
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<tr>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>Toxigenic <em>C. difficile</em></td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>Hypervirulent <em>C. difficile</em></td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>Inhibited</td>
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</tbody>
</table>

*Human Genetic Signatures*
Summary

• The HGS EasyScreen C. difficile assays sensitively and specifically detects toxigenic and/or hypervirulent *C. difficile*
  – <3 hours
  – Validated on multiple DNA extraction/purification platforms
  – Compatible with multiple real-time PCR machines
  – Quick, 10mins hands-on time if using automated purification platforms.

• Only commercially available test that incorporates gyrA as marker for hypervirulence
  – Reduce false positive identification of potentially hypervirulent strain
Team and Acknowledgements

- HGS
  - Dr Douglas Millar*
  - Dr John Melki*
  - Ms Jiny Nair
  - Dr Kiran Kaur
  - Dr Nicola Boulter

- Mr Peter Huntington* (Abstract 624) from PaLMS
- Mr Thomas Karagiannis* from PaLMS, RNSH
- Prof. Tom Riley*, UWA
- Prof. William Rawlinson* from SEALS, PoWH