Glutamate dehydrogenase (GDH) is Highly Conserved Among *Clostridium difficile* Ribotypes


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**Background:**

Immunoadsorbents for GDH, a metabolic enzyme encoded by *gluD*, are used to detect *C. difficile* in diarrhoeal fecal samples. The diagnostic utility of GDH requires isolates of all ribotypes to react in such assays. Recent reports (Tenover et al., 2010; Carroll, 2011) claim commercial GDH assays miss some ribotypes, in particular ARL 002, ARL 027, and ARL 106. It has been proposed that these ribotype-specific false negative results can be explained by *gluD* negative ribotypes and/or antigenic variation in GDH.

**Aims:**

1. To amplify the *gluD* gene of 84 *C. difficile* ribotypes by PCR and screen immunoreactivity in GDH immunoassays.
2. To quantify in vitro GDH production of all 88 ribotypes.
3. To sequence *gluD* and compare predicted amino acid sequences.

**Materials and methods**

*C. difficile* isolates: 84 ribotypes (including ARL 001, 002, 003, 012, 014, 017, 027, 033, 036, 043, 053, 054, 078, 106, 110, 126, and 154) were subcultured to 5mL Brain Heart Infusion (BHI) broth tubes (Anaerobe Systems, Morgan Hill, CA) and incubated anaerobically at 37°C for 72 hours.

GDH Assays: 72 hour BHI broth cultures were tested in the *Complete* DIFF QUIK CHEK® and *Complete* DIFF QUIK CHEK COMPLETE®, and the C.DIFF CHEK™-60 tests (TechLab®, Inc., Blacksburg, VA). Bacterial DNA: DNA was extracted using the IQAGEN DNA Mini Kit (Valencia, CA). Eluted DNA was stored at −10°C until ready for analysis.

PCR: *gluD*, encompassing the glutamine and NAD binding domains, from 27 ribotypes was amplified by PCR. See Table 1.

Sequencing: PCR products were sequenced by VBI (Blacksburg, VA) and then analyzed using ClustalW2 (EMBL-EBI).

Toxins and GDH Immunoadsorbent: A quantitative immunoadsorbent was used to detect the amount of GDH. Broth cultures were diluted, centrifuged, and filtered. The filtrate was incubated for 30 minutes with specific capture antibodies. A second 30 minute incubation with detection antibodies was done. A fluorescence detection was performed prior to analysis.

**TABLE 1: PCR conditions and primers for *gluD* amplification**

<table>
<thead>
<tr>
<th>Ribotype</th>
<th>Gene amplified</th>
<th>Forward Primer (5’ to 3’)</th>
<th>Reverse Primer (5’ to 3’)</th>
<th>Thermocycling Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARL 001</td>
<td>ARL 001</td>
<td>ARL 001 F</td>
<td>ARL 001 R</td>
<td>94°C 30 sec 60°C 1 min</td>
</tr>
<tr>
<td>ARL 002</td>
<td>ARL 002</td>
<td>ARL 002 F</td>
<td>ARL 002 R</td>
<td>94°C 30 sec 60°C 1 min</td>
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<tr>
<td>ARL 027</td>
<td>ARL 027</td>
<td>ARL 027 F</td>
<td>ARL 027 R</td>
<td>94°C 30 sec 60°C 1 min</td>
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<tr>
<td>ARL 106</td>
<td>ARL 106</td>
<td>ARL 106 F</td>
<td>ARL 106 R</td>
<td>94°C 30 sec 60°C 1 min</td>
</tr>
<tr>
<td>ARL 154</td>
<td>ARL 154</td>
<td>ARL 154 F</td>
<td>ARL 154 R</td>
<td>94°C 30 sec 60°C 1 min</td>
</tr>
</tbody>
</table>

**FIGURE 2: Phylogram for *gluD* predicted amino acid sequence alignment**

**FIGURE 3: Differences in *gluD* DNA sequences**

**Results:**

1. *gluD* gene was amplified from all 84 ribotypes, including 002, 027 and 106; there were no apparent size differences between all replicates, which ampiclon. All ribotypes were positive in the GDH immunoassays.

2. All ribotypes expressed readily detectable levels (>12.5 ng/mL) of GDH in vitro.

3. All 27 predicted amino acid sequences generated from *gluD* DNA sequences were identical with the exception of ARL 054.

**Conclusions:**

Because all 104 isolates of 84 ribotypes assayed contain *gluD* gene, and each reacted in all three GDH immunoassays, our results suggest that *gluD* negative ribotypes do not exist; nor cause ribotype-specific false negative results. All isolates made easily detectable levels of GDH in vitro. Furthermore, no variation in the size of the *gluD* ampiclon nor the predicted amino acid sequence it generates was observed; suggesting that there is no antigenic variation in GDH.

**References:**


*3582.0*