**E. coli ID™ – DNA Fingerprinting of E. coli**
(Discriminant Analysis of Ribotype Profiles of E. coli)

Submitter: XYZ Municipal Water Plant
Submitter #: ABCDE
Source Molecular #: SM 0111
Sample Received: January 3rd, 2011
Date Reported: January 10th, 2011

<table>
<thead>
<tr>
<th>Fecal Coliform&lt;sup&gt;5&lt;/sup&gt; mpn&lt;sup&gt;5&lt;/sup&gt;/100 ml</th>
<th>E. coli Isolate # &lt;br&gt;(5 colonies of cultured E. coli were analyzed)</th>
<th>Probable Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2,400</td>
<td>1</td>
<td>Animal</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Animal</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Indeterminate</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Animal</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Human</td>
</tr>
</tbody>
</table>

* mpn = most probable number of fecal coliforms in 100mL of sample after 20 hrs of cultivation at 44.5°C.

**Laboratory Comments**

Five E. coli DNA fingerprints were compared with an internal library of E. coli strains to determine whether they were from an animal or a human source (or both). The information provided should only be considered a preliminary source indicator. The client must run additional tests such as the E. coli Comparison ID™ test to confirm animal / indeterminate sources and the Human Enterococcus ID™ test to confirm the presence of human sources.

As shown in the above table, the DNA fingerprints of 4 colonies of E. coli cultured from the water sample statistically matched both human and animal sources when compared to a database of known source DNA fingerprints. One of the E. coli isolate DNA fingerprints was classified as indeterminate. The probable source of this E. coli isolate can either be human or animal.
DNA Fingerprinting Method Explanation

E. coli were enumerated by taking plates that are positive for fecal coliforms, transferring the membrane filter to EC with MUG media (Difco), and incubating for an additional 24 hours at 37°C. Colonies that fluoresced under UV light were counted as E. coli and isolated for ribotyping.

Ribotyping of E. coli isolates was accomplished by the method of Parveen et al. (1999). Chromosomal DNA was extracted from E. coli isolates and digested with HindIII. Fragments were separated by agarose electrophoresis. The DNA was then transferred and fixed to a Zeta-probe membrane. A cDNA probe complementary to the E. coli 16S and 23S rDNA was labeled with digoxigenin-dUTP and was used to probe the membranes. The resulting genetic fingerprint was translated to a binary code based on the presence and absence of predetermined bands. The resulting binary code was then analyzed by discriminant analysis using Bionumerics software against a library of known source isolates—similar to the method elaborated in Scott et al. (2003).

DNA Fingerprinting Theory Explanation

After cultivating E. coli from the submitted sample, one or more E. coli isolates are selected. Isolates are clusters of E. coli colonies on an agar plate. A DNA fingerprinting analysis called ribotyping is performed on each E. coli isolate selected. This genetic fingerprint comes from genes that code for ribosomal ribonucleic acids (rRNA) of E. coli. Ribosomal RNA together with various proteins makes up the cell structure called a ribosome.

The ribosome is the cell structure where proteins are manufactured. In order to produce proteins, the messenger RNA and the amino acids are transferred to the ribosome. As the ribosome moves down the messenger RNA, it places the correct amino acid in the growing protein. It has been shown that looking at small differences in the DNA that code for these 16S and 23S rRNA’s help identify different strains of E. coli.

Ribosomal genes are also known to be highly conserved in microbes, meaning that the genetic information coding for rRNA will vary much less within bacteria of the same strain than it will between bacterial strains. This characteristic allows for a greater ability to distinguish between different bacterial strains.

In ribotyping, restriction enzymes are used to cut the genes coding for rRNA into pieces, and electrophoresis separates the pieces by size through a gel. Genetic probes then visualize locations of different-size fragments of DNA in the gel, which appear as bands. The banding pattern of DNA fragments corresponding to the relevant rRNA is known as the ribotype. The banding patterns are compared to a database of other E. coli strains and matched for each determined strain. If the client submits fecal samples, then banding patterns are also investigated between the fecal samples and blind samples submitted.


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