Evaluation of Radioactive and Nonradioactive Gene Probes and Cell Culture for Detection of Poliovirus in Water Samples

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Poliovirus and other enteric viruses can be transmitted through the fecal-oral route. Waterborne disease outbreaks due to viral agents in potable water and improperly treated, sewage-contaminated water have been reported (1, 3, 5, 7). Currently, most treated waters and potable waters are not routinely assayed for enteric viruses because of the limitations of available methods. Cell culture, which until recently has been accepted as the standard method, has several drawbacks including the inability to distinguish between viral types, a time delay of 3 to 21 days, the high cost, and the lack of sensitivity (6). Hence, the evaluation of water quality relies on indicator bacteria as predictors of enteric viruses. Unfortunately, enteric viruses are less susceptible than coliforms to inactivation from the environment and disinfection procedures such as chlorination (10).

Alternative molecular techniques which have been previously confined to clinical or genetic laboratories permit the detection of minute quantities of nucleic acid (9). The properties of these probe assays, such as rapid and sensitive results, relative ease of use, and overall lower cost, may permit them to be used as tools for monitoring water for the presence of contamination by enteric viruses.

Buffer green monkey (BGM) cells were used for the propagation of poliovirus type 1-LSc. Virus was harvested after a 2-day incubation, and complete cytopathic effect was observed. Virus was quantified by serial-dilution plaque assay on 25-cm² confluent monolayers of BGM cells (2). Agar overlay was prepared with 1% flask agar, 5% fetal bovine serum, and medium 199 (Sigma, St. Louis, Mo.).

Collection sites were chosen to provide a variety of sample types (see Table 2). Eighty gallons (303 liters) of water was collected, and potential viruses were harvested by the method of Sobsey (9). Viruses were further concentrated by flocculation to a final volume of 30 ml by the technique of Katzanelson et al. (4).

Viral nucleic acid was released from the protein coat by the methods of Richardson et al. (8), except that samples were returned to room temperature with the aid of an ice bath prior to being spotted onto nylon-backed membranes. For nonradioactive probes, 1 ml of the sample was passed through a Bio-Rad bio-dot apparatus onto a GeneScreen (Dupont, Boston, Mass.) hybridization membrane in triplicate. For isotopic probes, GeneScreen Plus (Dupont) was substituted.

A single-stranded (ss) [32P]RNA probe was made by taking the 1,174-bp fragment from the PstI cut of poliovirus cDNA and inserting it into plasmid pGEM-3Z to form plasmid pGEM-3Z/PV 1174. Isotopic RNA transcripts were then made with SP6 RNA polymerase.

The first nonisotopic DNA probe was made by adding a poly(T) tail to the probe sequences by using terminal transferase as specified by the manufacturer of the Biobridge Labeling Kit (Enzo Diagnostics, Inc., New York, N.Y.). The sequences were generated by digesting plasmid PV104 with DNase for 10 min at 65°C. Hybridization to a biotinylated poly(A) sequence and subsequent detection were also performed as specified by the manufacturer.

In the second probe assay, plasmid PV104 was sonicated to shear DNA into pieces 50 to 300 bp in length. Horseradish peroxidase was directly conjugated to the DNA by using a proprietary method (DIGENE Diagnostics, Inc., Silver Spring, Md.). For colorimetric results, the membrane was allowed to incubate for 15 min in 15 mM Tris-HCl (pH 7.6)-0.05% NiCl2-0.5 μg of diaminobenzidine per ml. For chemiluminescence results, the membrane was incubated for 1 min in luminol provided in the ECL Western immunoblotting detection kit (Amersham Corp., Amersham, England) and allowed to expose Hyperfilm-ECL (Amersham) high-performance luminescence detection film for 1 min.

In the third nonisotopic assay, plasmid PV104 was labeled with the Genius 1 kit (Boehringer Mannheim, Indianapolis, Ind.). Digoxigenin-labeled dUTP was incorporated into DNA via random priming. Detection was also performed as

### TABLE 1. Comparison of sensitivities of each assay for detection of laboratory-produced poliovirus

<table>
<thead>
<tr>
<th>Method</th>
<th>Amt of cDNA (pg) at detection limit</th>
<th>Detection of virus a at following dilution of original viral suspension:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10⁻¹</td>
</tr>
<tr>
<td>Plaque assay</td>
<td>NA*</td>
<td>+</td>
</tr>
<tr>
<td>ss [32P]RNA</td>
<td>0.01</td>
<td>+</td>
</tr>
<tr>
<td>Digoxigenin</td>
<td>0.1</td>
<td>+</td>
</tr>
<tr>
<td>HRP</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>Biotin</td>
<td>100</td>
<td>+</td>
</tr>
</tbody>
</table>

* NA: not applicable.

**TABLE 1.** Comparison of sensitivities of each assay for detection of laboratory-produced poliovirus.

* Scale: 0, nondetectable; 1, barely visible; 2, clearly visible; 3, strong signal; 4, highest intensity.

* TNTC, too numerous to count.

* Number of FFU.

* HRP, horseradish peroxidase.
TABLE 2. Number of poliovirus detections at sample locations

<table>
<thead>
<tr>
<th>Sample location*</th>
<th>No. of samples positive/no. assayed:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell culture</td>
<td>[32P]DNA</td>
<td>ss [32P]RNA</td>
<td>Digoxigenin</td>
</tr>
<tr>
<td>A</td>
<td>1/4</td>
<td>1/4</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>B</td>
<td>1/7</td>
<td>2/7</td>
<td>2/7</td>
<td>2/7</td>
</tr>
<tr>
<td>C</td>
<td>0/5</td>
<td>0/5</td>
<td>2/5</td>
<td>2/5</td>
</tr>
<tr>
<td>D</td>
<td>0/7</td>
<td>1/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

* A, freshwater sample (Mill Pond); B, saltwater sample taken upstream from water treatment plant and downstream freshwater location (Town Landing); C, effluent from water treatment plant; D, saltwater sample taken downstream from water treatment plant (Painted Rock).

specified by the manufacturer, with the exception of prehybridization for 2 h instead of 1 h.

Table 1 demonstrates the sensitivities of the probe assays. The digoxigenin probe was consistently seen as the most sensitive nonisotopic system and so became the assay of choice for the environmental screening along with the ss [32P]RNA probe. Environmental samples with previously known cell culture and [32P]DNA probe results were coded and spotted onto GeneScreen and GeneScreen Plus membranes.

Comparison of the probe assays with cell culture for the detection of laboratory-grown poliovirus showed that cell culture was more sensitive than isotope-labeled and nonisotope-labeled nucleic acid probes. Digoxigenin was found to be 0.5 log unit less sensitive than the ss [32P]RNA but comparable to the randomly primed [32P]DNA (data not shown). No changes in sensitivity were observed between chemiluminescent and colormetric signals as a result of the extended blocking needed to reduce background associated with chemiluminescence to an acceptable level.

For the environmental samples (Table 2), a 100% correlation exists between the digoxigenin probe and ss [32P]RNA probe. The correlation between these two techniques and the [32P]DNA-based probe was 82%. Samples which were positive by cell culture yielded stronger signals in all three probe assays than did samples which were positive by one or more probe assays but negative by tissue culture.

Comparison of the digoxigenin and isotopic assays with cell culture in the evaluation of environmental samples showed that all probe assays detected poliovirus with greater frequency than cell culture did. This would indicate that, although nucleic acid hybridization assays lack similar sensitivity to cell culture for detection of laboratory-produced virus, they do possess the sensitivity needed for detection of contaminating enteric viruses in water samples. Since particle-to-PFU ratios have been reported to be as high as 1,000:1, and since these particles often contain a portion of the genome, they would be detected by gene probes but not by cell culture. Although one may argue that the presence of noninfectious viruses does not present a health risk, detection of the particle may be able to act as an indication of fecal pollution.

The digoxigenin probe was found to be nearly as sensitive as ss [32P]RNA probes, yet results can be obtained in 24 h, compared with 3 days for isotopic probes. Development of a rapid assay, like that used for detection of indicator organisms, will be key in the establishment of a virus-monitoring program. In addition, use of nonisotopic labels avoids the inherent dangers and disposal problems of radioactivity.

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REFERENCES