By analyzing DNA from isolates, the contamination of source and/or drinking water by Cryptosporidium oocysts and Giardia cysts could be traced back to animal or human origin (1-4). We carried out the nested PCR assay using DNA templates extracted from preparations for routine microscopic test (5).

The suspensions of Cryptosporidium oocysts and Giardia cysts were diluted and mixed adequately to contain 5 to 20 (oo)cysts in 50 μl. Each suspension was stained with FITC-labeled monoclonal antibody (EasyStain C/G FITC, Biotechnology Frontiers, Sydney, Australia) for 15 min, followed by DAPI for 5 min. In order to eliminate possible interference at downstream steps, we prepared a square PTFE membrane filter, and a total volume of suspension was applied evenly to the membrane filter. After observing and counting (oo)cysts under the fluorescence and differential interference contrast microscope, the coverslip was taken off and mounting medium on the back of the coverslip was wiped away with the retrieved membrane filter so as not to leave any (oo)cysts, and the membrane filter was placed into a 1.5-ml screw-cap microtube. An extraction of DNA from (oo)cysts was performed with a commercial DNA extraction kit (DNeasy Tissue Kit, QIAGEN, Hilden, Germany) according to the manufacturer’s protocol, with the addition of the following two steps. Initially, 180 μl of ATL buffer was added to the membrane filter in the screw-cap microtube and vigorously mixed; a freezing (in liquid nitrogen for 3 min)-thawing (in boiling water for 3 min) cycle was then repeated 15 times. Subsequently, a sonication step using an output at 200 W (Bioruptor UCD-200T, CosmoBio, Tokyo, Japan) was performed for 15 min. We employed the nested PCR assay for Cryptosporidium oocysts described by Xiao et al. (1-3) and that for Giardia cysts reported by Sulaiman et al. (4,6) with modifications for the volume of PCR reaction mixtures (50 μl) and the volume of templates (10 μl in primary PCR and 5 μl in nested PCR for both Cryptosporidium and Giardia). The cycles in primary and nested PCR amplification for Cryptosporidium were 45 and 40, respectively. For Giardia PCR, the annealing temperature was adjusted at 55°C to prevent nonspecific amplification.

The numbers of stained (oo)cysts detected on the membrane filter after filtration are summarized in Table 1. The minimal number of oocysts necessary to give a positive PCR result from oocysts on the membrane filter was 6, while no PCR products were detected from the preparations containing more than 10 oocysts (Table 1). The reported detection limit of the nested PCR assay using oocysts on polycarbonate filters has been two to three, and this previously reported method consistently amplified DNA derived from preparations containing more than 5 oocysts (7). As for the detection limit of Giardia cysts, our method detected a PCR band from the preparation containing 6 cysts with low reproducibility (Table 1). There might be several explanations of why the present method required more oocysts than the original method; one possibility is that some crude DNA was left on the PTFE membrane filter.

Although an additional PCR trial is necessary to improve the amplification of target molecules, it is feasible, we believe, to make use of preparations after the microscopic test as a source of DNA for further genetic analysis of the microbes detected.
We are grateful to Dr. Kenji Yagita, National Institute of Infectious Diseases, Tokyo for the distribution of *Cryptosporidium* oocysts and *Giardia* cysts.

**REFERENCES**


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Table 1. Number of preparations containing (oo)cysts on PTFE filters

<table>
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<th>No. of (oo) cysts</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>13</th>
<th>14</th>
<th>16</th>
<th>22</th>
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<tr>
<td>Cryptosporidium</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2(1)*</td>
<td>1(1)</td>
<td>2(1)</td>
<td>3(2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Giardia</td>
<td>2</td>
<td>1(1)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2(1)</td>
<td>1(1)</td>
<td>2</td>
<td>1(1)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*: Number in parenthesis of preparations positive for PCR amplification.