Comparison of Real-Time PCR Protocols for Differential Laboratory Diagnosis of Amebiasis

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Specific identification of Entamoeba spp. in clinical specimens is an important confirmatory diagnostic step in the management of patients who may be infected with Entamoeba histolytica, the species that causes clinical amebiasis. Distinct real-time PCR protocols have recently been published for identification of E. histolytica and differentiation from the morphologically identical nonpathogenic Entamoeba dispar. In this study, we compared three E. histolytica real-time PCR techniques published by December 2004. The limits of detection and efficiency of each real-time PCR assay were determined using DNA extracted from stool samples spiked with serially diluted cultured E. histolytica trophozoites. The ability of each assay to correctly distinguish E. histolytica from E. dispar was evaluated with DNA extracted from patients’ stools and liver aspirates submitted for confirmatory diagnosis. Real-time PCR allowed quantitative analysis of the spiked stool samples, but major differences in detection limits and assay performance were observed among the evaluated tests. These results illustrate the usefulness of comparative evaluations of diagnostic assays.

Clinical features of amebiasis, caused by the protozoan parasite Entamoeba histolytica, range from asymptomatic colonization to amebic dysentery and invasive extraintestinal amebiasis, most commonly in the form of liver abscesses (24). The World Health Organization estimates that amebiasis is one of the three most common causes of death from parasitic disease, responsible for up to 100,000 deaths annually (30). The disease is spread primarily by food or water contaminated with cysts but may also be transmitted from person to person. It is highly prevalent in regions of the world where personal hygiene and/or sanitation are insufficient.

Examination of stained stool smears is used routinely in clinical laboratories to differentiate E. histolytica from non-pathogenic intestinal amebas, such as Entamoeba coli, Entamoeba polecki, and Entamoeba hartmanni. However, this gold standard method cannot differentiate E. histolytica from the morphologically identical E. dispar, which occurs worldwide (8). E. dispar is a harmless commensal protozoan, and its presence in clinical specimens does not justify treatment (30). Thus, misidentification of E. histolytica-associated disease may occur if the diagnosis is based solely on examination of smears (29). For final confirmatory identification of intestinal amebiasis, molecular methods or immunologic assays for detection of E. histolytica antigens are needed (21). Currently, the only commercially available antigen test for specific detection of E. histolytica (the histolytica II test from TechLab) is recommended for use exclusively with fresh stool samples, since storage or use of preservatives destroys the antigen.

For diagnosis of extraintestinal amebiasis, the laboratory methods are even more limited. Detection of amebas by microscopy is often unsuccessful (32). Although acceptable results with extraintestinal specimens have been obtained with the TechLab II antigen test (14, 22), this test is designed and marketed for examination of stool specimens only.

PCR, including real-time PCR, has provided means to identify E. histolytica in a variety of clinical specimens, including stools, tissues, and liver abscess aspirates (24). Several PCR assays designed for differential detection of E. histolytica and E. dispar have been developed. Most of them target either the small-subunit rRNA (18S rRNA) gene (5, 10, 15, 18) or species-specific episomal repeats (1, 19, 21). These targets are present on multicopy, extrachromosomal plasmids in the amebas (3). The sensitivity and specificity of PCR assays exceed what can be accomplished with microscopy and are comparable to those of the antigen test (13, 16, 17, 20, 23).

Real-time PCR is a very attractive methodology for laboratory diagnosis of infectious diseases because of its features that eliminate post-PCR analysis, leading to shorter turn-around times and minimized risk of amplicon contamination of laboratory environments. This represents obvious advantages in diagnostics, as amplicon contamination has been reported to be the most frequent cause of false-positive results in PCR amplification (31). In addition, real-time PCR is a quantitative method and may allow the determination of the number of parasites in various samples (2). Although not relevant for estimating the parasite burden in amebiasis patients (the parasite content can vary tremendously between, or even within, specimens from the same patient), quantitative measures can

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followed by modifications: (i) liver extract (Oxoid), 15 g/liter, was substituted for strain HM1 was grown in Diamond’s TYIS-33 medium (9) at 37°C with the eight diluted samples in each series. This was repeated four more times, an aliquot was serially diluted to 10−5 to 3.0 × 107 cells/ml, creating three identical dilution series /

harvested batch were used to produce a set of 120 quantification standards: each concentration of trophozoites was calculated. Three 200-

/Cryptosporidium hominis, and


TABLE 1. Primers and probes used in the real-time PCR assays compared

<table>
<thead>
<tr>
<th>Assay</th>
<th>Gene target</th>
<th>Amplicon size (bp)</th>
<th>Primer or probe</th>
<th>Sequence (5′ to 3′)</th>
<th>Nucleotide positions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional PCR adapted for SYBR</td>
<td>18S rRNA</td>
<td>877</td>
<td>PSP6</td>
<td>GGCACATTTATGAAATGTAGG</td>
<td>200–223</td>
<td>5</td>
</tr>
<tr>
<td>Green real-time PCR</td>
<td>18S rRNA</td>
<td>878</td>
<td>PSPF</td>
<td>CTGACATGACACCAATGCTTCTC</td>
<td>1076–1052</td>
<td>5</td>
</tr>
<tr>
<td>LightCycler</td>
<td>18S rRNA</td>
<td>307</td>
<td>NPS3</td>
<td>GGCACATTTATGAAATGTAGG</td>
<td>200–223</td>
<td>5</td>
</tr>
<tr>
<td>TaqMan 1</td>
<td>18S rRNA</td>
<td>231</td>
<td>NPS3</td>
<td>CTGACATGACACCAATGCTTCTC</td>
<td>1076–1052</td>
<td>5</td>
</tr>
<tr>
<td>TaqMan 2</td>
<td>Episomal repeats</td>
<td>83</td>
<td>Ehd-239F</td>
<td>ATGTGCTGGTGCCTTCATCTA</td>
<td>260–239</td>
<td>28</td>
</tr>
<tr>
<td></td>
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</table>

# MATERIALS AND METHODS

Cultured Entamoeba histolytica trophozoites. Entamoeba histolytica ATCC strain HM1 was grown in Diamond’s TYIS-33 medium (9) at 37°C with the following modifications: (i) liver extract (Oxoid), 15 g/liter, was substituted for casein digest peptone (BBL), (ii) yeast extract was used at 25 g/liter, and (iii) the vitamin mixture consisted of NCTC 109 with added vitamins B12, D, and l-thiouric acid and Tween 80 solution. Cultured E. histolytica trophozoites were harvested, centrifuged, and resuspended in phosphate-buffered saline to 0.2 × 106 to 3.0 × 108 cells/ml. A 1-μl aliquot of this harvested batch was placed in a counting chamber (Hauser Scientific Company, Horsham, Pa.), and the concentration of trophozoites was calculated. Three 200-μl aliquots of the same harvested batch were used to produce a set of 120 quantification standards: each aliquot was serially diluted to 10−5 cell/ml, creating three identical dilution series with eight diluted samples in each series. This was repeated four more times, resulting in five batches of three dilution series each. A volume of 200 μl of each diluted sample was used to spike 200 μl parasite-free stools, which were then subjected to DNA extraction (see below). A single stool sample, obtained from a volunteer with no symptoms of intestinal infection, was used in all spiking experiments. Prior to the spiking procedure, this stool was evaluated for the presence of parasites by microscopic examination of wet mounts and for E. histolytica and E. dispar using the conventional PCR described below.

**Clinical specimens.** A total of 51 clinical specimens (42 stools and 9 liver abscess specimens) were used to evaluate the real-time PCR tests. The specimens had been submitted to CDC from state health departments in the United States for confirmatory diagnosis during the period from December 2003 to February 2005. Thirty-eight specimens were from patients with suspected amebiasis, collected from United States civilians with a travel history outside of the United States, immigrants, and refugees; of these, 29 were stools positive for E. histolytica/E. dispar by microscopy before submission to CDC, and nine were liver aspirates from nine patients clinically suspected to have amebic liver abscesses. These 38 specimens were tested for the presence of amebas by conventional PCR, using primers PSP5/PSP3 and NPS5P/NPS3 (5), upon receipt at CDC. The remaining 13 samples were stools containing other intestinal parasites, as confirmed by conventional PCR and DNA sequencing: two samples each of Entamoeba invadens, Encephalitozoon intestinalis, Enterocytozoon bieneusi, and Cyclospora cayetanensis; one sample each of Entamoeba coli, Entamoeba chitoni, Encephalitozoon cuniculi, Cryptosporidium parvum, and Cryptosporidium hominis. DNA extraction. Total genomic DNA was extracted from spiked stools and clinical samples using a modification of the FastDNA method (Q-Biogene, Carlsbad, Calif.) as previously described (6). DNA was extracted from 300 μl of each clinical sample. Samples were disrupted in the FP120 cell disruptor instrument at a speed of 5.5 for 10 seconds. Potential inhibitors were removed by further purification with the QIAquick PCR purification kit (QIAGEN Inc., Valencia, Calif.) according to the manufacturer’s instructions. Purified DNA was stored at 4°C until it was used in PCRs.

**PCR amplification.** All PCRs were performed using commercially available reagents that included a thermostable DNA polymerase, deoxynucleoside triphosphates, MgCl2, and other salts and buffering agents necessary for optimum performance. One microliter of template DNA was added to each reaction mixture, and the total volume was 20 μl in all assays. Conventional PCR, using previously published primers for E. histolytica detection (5), was employed to define the detection limit of the assay. Cycling was carried out in a GeneAmp 9700 PCR thermal cycler (Applied Biosciences, Foster City, Calif.). The LightCycler assay was performed on LightCycler 2.0 (Roche Diagnostics, Indianapolis, Ind.), whereas all other real-time PCR assays were performed on Mx3000P (Stratagene, La Jolla, Calif.). Details about the assays are outlined in Table 1.

* U, 5-propyne-2′-deoxyuridine. This chemistry mimics the effect on hybridization of the minor-groove binding protein in the so-called MGB probes. LC640, LightCycler Red 640; FAM, 6-carboxyfluorescein; BHQ1, Black Hole Quencher 1; HEX, hexachlorofluorescein; p, phosphate.

* Specific for E. histolytica.

* Specific for E. dispar.

* Generic for E. histolytica/E. dispar.
The variation in \(C_t\) values between samples containing the same parasite concentration was mainly due to variable DNA concentrations in the samples, as explained in Results. Differences between samples were estimated in quantification experiments using the DNA quantification standards. SYBR Green assay \(C_t\) values of the standard DNA samples were obtained with DNA extracted from stools containing \(10^7\) to \(10^{-1}\) \(E.\) histolytica trophozoites per ml. Each sample was tested three times, and the resulting \(C_t\) values were plotted against the trophozoite concentration. The line corresponds to a linear regression of all values. The variation in \(C_t\) values between samples containing the same parasite concentration was mainly due to variable DNA concentrations in the samples, as explained in Results.
real-time PCR assays, but it still had approximately 10-fold-higher sensitivity than conventional PCR (Table 4).

Regression analysis identified variable linear ranges for the assays, with the TaqMan assays having the largest possible linear range of 8 orders of magnitude (Fig. 2 and Table 4). The slopes of the linear parts of the standard curves in Fig. 2 were used to estimate the amplification efficiency. A slope of −3.3 translates into 100% efficiency, which in practice means that the number of amplicon copies doubles for each amplification cycle. By using this criterion, only the TaqMan assay targeting the 18S rRNA demonstrated 100% efficiency (Table 4).

The last two columns in Table 4 provide a rough estimate of the time and cost involved in running each evaluated real-time PCR assay. The SYBR Green assay and the LightCycler assay used species-specific primers to distinguish E. histolytica from E. dispar. Thus, two separate reactions had to be run in parallel for each sample, resulting in slightly longer set-up times than for the TaqMan assays. In addition, the LightCycler requires the reactions to be run in special glass capillaries, which are expensive and fragile, requiring extra care to work with. On the other hand, the LightCycler thermocycler has very fast ramping of the temperature, leading to shorter cycle times. Due to the high cost of fluorescent probes, the probe-free SYBR Green assay was the least expensive. However, because it was based on amplification of a large amplicon size, it required long cycling times, making it more time-consuming than the other real-time assays.

**Specificity of evaluated real-time PCR tests.** The abilities of the tests to accurately differentiate E. histolytica from E. dispar were determined using 29 stool and 9 liver aspirate specimens submitted for confirmatory diagnosis at CDC. These samples had been previously tested for amebiasis by conventional PCR with primers PSP3/PSP5 and NPSP3/NPSP5 (i.e., the same primers used in the SYBR Green assay). This had confirmed 10 of these samples as positive for E. histolytica (4 of the liver aspirates and 6 of the stool specimens), 16 of the stools as positive for E. dispar, and 1 stool as positive for both species. All 38 samples were reevaluated in this study, using the four real-time PCR assays, and the results are displayed in Tables 5 and 6. To ensure the accuracy of species determination, all samples that were positive for amebas in any of the evaluated tests were also amplified with primers Ehv-88R and Eh-Ed-AS25, and the amplicons were sequenced (Tables 5 and 6). The SYBR Green assay reported seven of the initially positive samples as negative for amebas. This could have been associated with long-term storage and subsequent degradation of nucleic acids, bringing the concentration of the targets below the detection limit of the SYBR Green assay before real-time PCR was performed. On the other hand, the probe-based assays (i.e., the LightCycler and the two TaqMan assays) confirmed the initial PCR results in all 27 positive samples (23 stools and 4 liver aspirates), and in addition detected amebas in four samples (3 stools and 1 liver aspirate) that had been negative in the initial PCR (Tables 5 and 6). One of the latter stool samples had been stored for some time before arriving at the CDC. The other two stools were from patients with suspected amebiasis based on microscopic findings in previous samples. The liver aspirate came from a person with a history of travel to countries where E. histolytica infections are endemic. Although confirmed to contain E. histolytica by sequencing, these four samples had very low parasite content, as judged by consistently high Ct values in the real-time PCR (Ct = 34 or above, depending on sample and assay). This corresponds to around 10 trophozoites or less per ml, which can explain the negative results in the conventional PCR and the SYBR Green assay.

The TaqMan targeting episomal repeats (TaqMan 2) and the LightCycler assay gave mixed results for three and five

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**TABLE 3. Statistical analysis of the quantification standards as evaluated in the SYBR Green assay**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value at indicated cell count/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^0</td>
</tr>
<tr>
<td>Avg C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>0.5 (±2.2)</td>
</tr>
<tr>
<td>No. of C&lt;sub&gt;t&lt;/sub&gt; values</td>
<td>45 (3 tests at 15 samples each)</td>
</tr>
<tr>
<td>C&lt;sub&gt;t&lt;/sub&gt; intervals</td>
<td>16.5–24.3</td>
</tr>
<tr>
<td>Significance</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

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**TABLE 4. Performance characteristics of assays**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Limit of detection (cells/ml [±SD])</th>
<th>Linear range (cells/ml)</th>
<th>Slope (efficiency)</th>
<th>Relative cost&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time&lt;sup&gt;b&lt;/sup&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional PCR</td>
<td>119 (±890)</td>
<td>10&lt;sup&gt;-3&lt;/sup&gt;–10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>−4.2 (72%)</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>SYBR Green</td>
<td>17 (±57)</td>
<td>10&lt;sup&gt;-2&lt;/sup&gt;–10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>−3.5 (90%)</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>LightCycler</td>
<td>4 (±14)</td>
<td>10&lt;sup&gt;-4&lt;/sup&gt;–10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>−3.3 (100%)</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>TaqMan 1</td>
<td>1 (±4)</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;–10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>−3.5 (91%)</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>TaqMan 2</td>
<td>0.5 (±1.2)</td>
<td>10&lt;sup&gt;-5&lt;/sup&gt;–10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>−3.5 (91%)</td>
<td>+</td>
<td>4</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Cost for equipment not included. The LightCycler assay requires a LightCycler thermocycler. The other real-time assays can be performed on less expensive real-time thermocyclers.

<sup>b</sup> Estimated time from reception of specimen to final result (2 h for DNA extraction is included).

<sup>c</sup> NA, not applicable.

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E. histolytica is the agent of human intestinal and extra-intestinal amebiasis, a parasitic infectious disease responsible for significant morbidity and mortality, mainly in developing countries. Accurate differentiation of the invasive E. histolytica from the morphologically identical commensal E. dispar is crucial to clinical management of patients and to epidemiologic investigation of outbreaks of amebiasis. Molecularly based differentiation of these two amebas has proven to be adequate for this purpose, and the use of real-time PCR should enhance it even further as a diagnostic application, as it allows fast and sensitive detection of E. histolytica in clinical specimens. This study provides a comparison of all real-time PCR procedures for laboratory diagnosis of amebiasis published through 2004 (4, 25, 27, 28). In addition, we evaluated a SYBR Green assay adapted from a conventional PCR technique published previously (5).

Compared to conventional PCR, real-time PCR has several advantages: not requiring postamplification analysis, which minimizes the risks for laboratory contamination (7); ability to differentiate between E. histolytica and E. dispar infections in a duplex profile (27, 28); and numerical results, which are easier to interpret than the visual examination of a stained gel from a conventional PCR. Nevertheless, real-time PCR is a costly procedure compared with morphological stool exams and antigen-based detection tests. Thus, poor regions of the world, where E. histolytica is most prevalent, will unfortunately be less likely to benefit from real-time PCR. Instead, this technique will be feasible primarily in clinical laboratories in developed countries that need to diagnose amebiasis in travelers and immigrants from regions of the world where E. histolytica is endemic.

An important aspect of real-time PCR is its enhanced sensitivity compared to conventional PCR. As expected, all real-time PCR assays in this study were more sensitive than the conventional PCR, a result that is in agreement with a recent study comparing a novel real-time PCR assay with conventional PCR for amebiasis (22).

The probe-based real-time PCR assays evaluated in this study were able to identify E. histolytica in four clinical samples with very low parasite concentrations, which the conventional PCR could not detect. The most sensitive real-time PCR assay tested was TaqMan 2, i.e., the one designed to amplify episomal-repeat regions (28). The calculated detection limit for this assay was 0.5 cells per ml of spiked stool, which would mean that samples containing only 0.1 cell (DNA was extracted from stool samples, respectively, that contained only E. dispar as judged by the other methods (Table 5). In addition, the LightCycler assay had problems with the standard DNA samples from cultured E. histolytica, which occasionally were detected as positive for E. dispar as well (data not shown). Attempts to reduce this problem by altering the PCR conditions were mostly unsuccessful, although a slight improvement in the LightCycler assay was seen when the concentration of oligonucleotides was decreased compared to the published protocol (data not shown). The sequencing analysis verified the results obtained with the 18S rRNA-targeting TaqMan assay (TaqMan 1) and did not support the mixed results produced by TaqMan 2 or the LightCycler assay.

**DISCUSSION**

E. histolytica is the agent of human intestinal and extra-intestinal amebiasis, a parasitic infectious disease responsible for significant morbidity and mortality, mainly in developing countries. Accurate differentiation of the invasive E. histolytica from the morphologically identical commensal E. dispar is crucial to clinical management of patients and to epidemiologic investigation of outbreaks of amebiasis. Molecularly based differentiation of these two amebas has proven to be adequate for this purpose, and the use of real-time PCR should enhance it even further as a diagnostic application, as it allows fast and sensitive detection of E. histolytica in clinical specimens. This study provides a comparison of all real-time PCR procedures for laboratory diagnosis of amebiasis published through 2004 (4, 25, 27, 28). In addition, we evaluated a SYBR Green assay adapted from a conventional PCR technique published previously (5).

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**DISCUSSION**

E. histolytica is the agent of human intestinal and extra-intestinal amebiasis, a parasitic infectious disease responsible

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**TABLE 5. Conventional and real-time PCR results for stool specimens submitted to CDC for confirmatory diagnosis**

<table>
<thead>
<tr>
<th>Methodology</th>
<th>E. histolytica</th>
<th>E. dispar</th>
<th>Mixed</th>
<th>Negative</th>
<th>Mixed</th>
<th>E. dispar</th>
<th>Negative</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Sequencinga</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>16</td>
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<tr>
<td>Conventional PCRb</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>SYBR Green</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
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<tr>
<td>LightCycler</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>TaqMan 1</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TaqMan 2</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

a DNA sequencing analysis was performed only to assure the results and not for comparison purposes.

b Results from confirmatory diagnosis, performed directly upon reception of the specimen at CDC.

c 13 of these were clinical specimens containing other parasites than E. histolytica or E. dispar; see “Clinical specimens” for details. They were included in this study as negative controls for the real-time PCR assays. Conventional PCR for ameba detection was not performed on these samples, as they were not from suspected amebiasis cases.
mixed sample as containing *E. dispar* only. The explanation for this is associated with the duplex assay profile of the latter TaqMan assay, which used the same primers for simultaneous amplification of both species. The overabundance of one species can mask the ability to detect a second species when the same amplification primers are shared. When presented with these circumstances, such duplex (or multiplex) assays that distinguish between targets only by different probes are not suitable for simultaneous detection of more than one microorganism.

Two of the assays could not reliably distinguish *E. histolytica* from *E. dispar*: the LightCycler assay and the TaqMan assay targeting episomal repeats (TaqMan 2). The LightCycler assay occasionally reported false-positive results for both *E. dispar* and *E. histolytica*-containing samples, including pure *E. histolytica* cultures. Furthermore, the false-positive results were not consistent but varied from run to run. This behavior clearly illustrates a lack of specificity of the primers. Thus, in our hands, the LightCycler assay was not considered specific enough to serve as a diagnostic tool for the main purpose of distinguishing *E. histolytica* from *E. dispar*. The TaqMan 2 assay produced false results with a few samples containing *E. dispar*. Two other publications have reported peculiar results concerning detection of *E. dispar* in conventional PCRs targeting these episomal-repeat sequences. Verweij and coworkers (26) were unable to detect one sample that was positive for *E. dispar* in two other PCR assays, concluding that the episomal-repeat region seemed to be absent in that particular *E. dispar* sample. A recent study (11) detected both *E. dispar* and *E. histolytica* in a liver pus sample, which must be a false result for *E. dispar*, since the species is not invasive. Thus, an explanation for the nonspecific results obtained with TaqMan 2 in this study may be that these target sequences are not as species specific as previously reported. Supporting this explanation is the presence of a sequence from an *E. dispar* strain in GenBank that is highly similar to the assumed *E. histolytica*-specific episomal repeat (accession number AJ306927). This calls for a reevaluation of the episomal repeats as targets for differential molecular diagnosis of amebiasis.

In conclusion, this work identified the TaqMan targeting the 18S rRNA gene as a superior real-time PCR assay for specific and quantitative diagnosis of amebiasis. The SYBR Green approach offered a good alternative to the TaqMan assay and may be especially attractive for those who already have the conventional PCR assay running and want to convert to the real-time format.

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The use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

**REFERENCES**


