Bacteriology

Five commercial DNA extraction systems tested and compared on a stool sample collection

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Abstract

In this study, 5 different commercial DNA extraction systems were tested on a stool sample collection containing 81 clinical stool specimens that were culture-positive for diarrheagenic Escherichia coli, Campylobacter jejuni, Salmonella enterica, or Clostridium difficile. The purified DNAs were analyzed by polymerase chain reaction (PCR) directed toward the relevant organisms. The results showed that conventional PCR combined with the extraction systems BioRobot EZ1 (Qiagen, Hilden, Germany), Bugs’n Beads (Genpoint, Oslo, Norway), ChargeSwitch (Invitrogen, Paisley, UK), QIAamp Stool Mini Kit (Qiagen), and 2 protocols (generic and Specific A) for EasyMag (BioMérieux, Marcy l’Etoile, France) were able to identify 89%, 62%, 85%, 88%, 85%, and 91%, respectively, of the pathogens originally identified by conventional culture-based methods. When TaqMan PCR was combined with the EasyMag Specific A protocol, 99% of the samples were correctly identified. The results demonstrate that the extraction efficiencies can vary significantly among different extraction systems, careful optimization may have a significant positive effect, and the use of sensitive and specific detection methods like TaqMan PCR is an ideal choice for this type of analysis.

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1. Introduction

Conventional diagnostics of gastrointestinal bacterial pathogens include culturing on selective media followed by biochemical characterization and serotyping. Such analyses have been the gold standard for many bacterial pathogens for several decades, and this is not without reason since they have allowed very specific characterization at species and subspecies level. During the last couple of decades, the development of molecular techniques and the huge expansion of DNA sequence data have opened the possibility of performing molecular identification of bacterial pathogens. The first step in molecular diagnostics is the extraction of intact template DNA, which may be generated by simple boiling of bacterial colonies from cultured samples or by direct DNA extraction from the sample. Depending on the selectivity and growth rate, culturing may be an advantage for a number of organisms but disadvantageous for slow-growing or fastidious organisms. These organisms may be easily detected by direct DNA extraction followed by polymerase chain reaction (PCR) that also has the benefit of detecting dead bacteria and bacteria prone to lose virulence traits upon culturing.

DNA extraction from stool specimens is particularly challenging because several different unfavorable substances may coextract and have an inhibitory effect on downstream PCRs (Abu Al-Soud and Radstrom, 1998; Kreader, 1996; Lantz et al., 1997; Monteiro et al., 1997) and the target DNA may be highly diluted in nontarget DNA of both bacterial and human origin (Weaver and Rowe, 1997; Wilson, 1997). Many different extraction methods, of both in-house (Argyros et al., 2000; Lou et al., 1997; Stacy-Phipps et al., 1995; Yang et al., 2007) and commercial types, have been developed and tested (Argyros et al., 2000; Okamoto et al., 2004; Søren Persson et al., 2008).
2. Materials and methods

2.1. Stool samples

A total of 81 culture-positive stool samples from diarrheagenic patients were collected randomly at our laboratory during the period of March to May 2006. These samples were grown on selective media and colonies were analyzed for enteropathogenic bacteria by the following methods: Campylobacter spp. was identified by growth on charcoal cefoperazone deoxycholate agar plates (SSI Diagnostica, Hillerød, Denmark) followed by PCR according to Persson and Olsen (2005). Clostridium difficile was isolated by culturing of boiled stool suspensions on cycloserine cefoxitin fructose agar plates (SSI Diagnostica) and toxigenic colonies were identified by PCR according to Persson et al. (2008). Diarrheagenic Escherichia coli, Shigella spp., and Salmonella spp. were isolated on SSI enteric medium plates (SSI Diagnostica) (Blom et al., 1999) and diarrheagenic E. coli was identified by PCR according to Persson et al. (2007).

To prepare a stool sample collection for DNA extractions, just enough sterile buffered saline (80 mmol/L NaCl, 50 mmol/L Na2HPO4, 10 mmol/L KH2PO4, pH 7.38) was added to each of the 81 stool samples containing 10 mmol/L EDTA to make the sample pipettable, was vortexed briefly, and was aliquoted into 10 tubes of 300 μL and stored at −80 °C.

2.2. DNA extraction

The following 5 commercial DNA extraction systems were included in the study: BioRobot EZ1 (Qiagen, Hilden, Germany), Bugs’n Beads (Genpoint, Oslo, Norway), ChargeSwitch (Invitrogen, Paisley, UK), EasyMag (bioMérieux, Marcy l’Etoile, France) (generic and Specific A protocol), and QIAamp Stool Mini Kit (Qiagen). The procedures on all extraction systems were done according to the recommendation of the manufacturers. For each extraction system, a new set of aliquots of the 81 stool samples were thawed and kept on ice until the start of the extraction procedure. Volumes (microliters) of (1) input stool sample, (2) elution buffer, (3) DNA template in 25 μL conventional PCRs, and (4) bovine serum albumin (BSA) (20 mg/mL) (Fermentas, Ontario, Canada) in 25 μL conventional PCRs for the 5 extraction systems were as follows: BioRobot EZ1 50/100/2/0.25, Bugs’n Beads 1.5/75/5/0.5, ChargeSwitch 75/150/2/0.25, EasyMag (generic) 17/55/2/0.25, EasyMag (Specific A) 33.3/110/2/0.25, and QIAamp Stool Mini Kit 25/200/5/0.5. The EasyMag Specific A protocol (in combination with the preextraction protocol for stool samples) and TaqMan PCR were performed at the Laboratory for Infectious Diseases in Groningen, The Netherlands (de Boer et al., 2010), and samples were shipped on dry ice between our 2 laboratories.

2.3. PCR methods

All DNA extractions were analyzed by conventional multiplex PCR (mPCR) methods directed toward diarrheagenic E. coli (Persson et al., 2007), Campylobacter coli and Campylobacter jejuni (Persson and Olsen, 2005), Salmonella enterica (Aabo et al., 1993), and Clostridium difficile (Persson et al., 2008) where PCR inhibition was evaluated by an internal amplification control directed toward a 1062-bp fragment of 16S rDNA. DNA extractions obtained by the EasyMag Specific A protocol were also analyzed by TaqMan mPCR methods directed toward VTEC (Schuurman et al., 2007b), EPEC (Friesema, de Boer, Duizer, et al., 2010, Etiology of acute gastroenteritis in children requiring hospitalization in the Netherlands, unpublished data), Shigella spp./IEC (Vu et al., 2004) Campylobacter jejuni (Best et al., 2003), Salmonella enterica (Malorny et al., 2004), and Clostridium difficile (de Boer et al., 2010). DNA extraction and PCR inhibition control for the TaqMan PCR were carried out by adding Phocine herpesvirus (PhHV) to the lysis buffer before the extraction procedure, as described in Schuurman et al. (2007a).

3. Results and discussion

Diagnostic PCR performed on DNA purified directly from stool samples is an attractive strategy because it may reduce the time of analysis and improve sensitivity and specificity. This strategy contains 2 key components: first, DNA extraction that is able to efficiently purify DNA out of stool specimens, that is, with a high DNA recovery and effective removal of PCR inhibitors; and second, PCR analyses that target the organisms of interest in a both specific and sensitive manner. The present study was undertaken to evaluate the purification efficiency of the 5 commercial DNA extraction systems: BioRobot EZ1,
Table 1

A total of 81 culture-positive stool samples analyzed by 6 different DNA extraction methods combined with conventional mPCR and TaqMan mPCR

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Conventional mPCR target</th>
<th>TaqMan mPCR target</th>
<th>Culture</th>
<th>Conventional mPCR</th>
<th>BioRobot EZ1</th>
<th>ChargeSwitch</th>
<th>EasyMag Specific A</th>
<th>TaqMan mPCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPEC</td>
<td>eae</td>
<td>escV</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>VTEC</td>
<td>vtx1/2, eae</td>
<td>vta1/2, escV</td>
<td>14</td>
<td>8</td>
<td>8</td>
<td>11</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>EIEC/Shigella spp.</td>
<td>ipaH</td>
<td>ipaH</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>JEO402-1</td>
<td>trtBCA</td>
<td>18</td>
<td>14</td>
<td>5</td>
<td>13</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>asp, hipO</td>
<td>mapA</td>
<td>17</td>
<td>15</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Toxigenic C. difficile</td>
<td>tcdA, tcdB, cdtA, cdtB</td>
<td>tcdA, tcdB</td>
<td>16</td>
<td>16</td>
<td>10</td>
<td>15</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Total positive: 81
% positive: 100
Stool volume per PCR (μL)\(^a\): 0.6, 0.1, 0.6, 0.6, 0.6, 1.5

\(^a\) Calculated from entered stool volume, dilution during the extraction procedure, and template volume per PCR.

EPEC = enteropathogenic E. coli; ETEC = enterotoxigenic E. coli; VTEC = verocytotoxin-producing E. coli; EIEC = enteroinvasive E. coli; eae = encoding intimin; estA = encoding heat stable enterotoxin A; eltA = encoding heat labile enterotoxin A; vtx1/2 = encoding verocytotoxin 1/2; ipaH = encoding invasive plasmid antigen H; JEO402-1 = Salmonella specific fragment; asp = encoding aspartokinase; hipO = encoding hippuricase; tcdA = encoding Clostridium difficile enterotoxin A; tcdB = encoding Clostridium difficile cytoxin B; cdtA and cdtB = encoding Clostridium difficile binary toxin (A and B component); escV = encoding pre (inner) membrane protein; trtBCA = encoding tetrathionate reductase; mapA = encoding outer membrane lipoprotein MapA.

Bugs’n Beads, ChargeSwitch, QIAamp Stool Mini Kit, and EasyMag (generic and Specific A protocols) and they were chosen to cover different technologies commercially available. EasyMag and BioRobot EZ1 are semiautomatic systems based on bacterial lysis followed by DNA extraction by magnetic beads with bound silica particles. Bugs’n Beads is a manual procedure that first isolates whole bacteria, by specific affinity of coated magnetic beads, followed by lysis and DNA extraction on the same beads. QIAamp DNA Stool Kit is a manual procedure that extracts DNA from lysed bacteria on spin columns with bound silica. ChargeSwitch is a manual procedure that extracts DNA from lysed bacteria by magnetic particles coated with the ChargeSwitch matrix.

All extraction systems were applied on identical aliquots from a stool sample collection containing 81 clinical stool specimens previously found positive for enteropathogenic E. coli (EPEC), verocytotoxin-producing E. coli (VTEC), enteroinvasive E. coli (EIEC), Shigella spp., Salmonella enterica, Campylobacter jejuni, or toxigenic Clostridium difficile by a combination of culture and PCR. The procedure of each extraction system was performed according to the recommendation of the manufacturer; and for the EasyMag system we had the opportunity to test 2 different protocols, a generic protocol and a protocol particularly developed for stool specimens that included an increased amount of magnetic silica, rigorous homogenization using a bead-beater during the lysis step, and elevated time and temperature at the elution step (Specific A protocol) (de Boer et al., 2010). The extracted DNA from each of the systems was subsequently analyzed by conventional mPCR, while TaqMan mPCR was applied only to DNA extracted by the EasyMag Specific A protocol. The results are listed in Table 1. The percentage of samples that were correctly identified by conventional mPCR varied from 62 to 91, while the EasyMag Specific A protocol combined with TaqMan mPCR identified all except 1 (99%) of the 81 samples correctly (the one sample had an inhibitory effect on the internal PCR control). The EasyMag Specific A extraction combined with conventional mPCR identified 74 samples correctly, which are 5 more samples than with the generic EasyMag protocol. This increase in correctly identified samples is therefore attributed to the differences listed above for the Specific A protocol and not related to the stool volume, since this is the same for both protocols. The EasyMag Specific A extraction combined with TaqMan mPCR identified 80 samples correctly. Compared to the Specific A protocol combined with conventional mPCR, this is an increase of 6 correctly indentified samples, which is therefore attributed to the TaqMan mPCR method. TaqMan PCR has often been reported having higher sensitivity than conventional PCR, which is mainly due to higher number of cycles performed in TaqMan PCR that is possible since unspecific amplification is less likely to interfere with the results because of the additional specificity obtained by the probe. In this study the TaqMan mPCRs were performed with 40 cycles compared to 35 cycles in the conventional mPCRs; and among all samples that were positive by TaqMan mPCR and either negative or positive by conventional mPCR (extracted by the EasyMag Specific A protocol), the threshold cycle values were between 29.1 and 37.4 (average, 33.4) and between 16.3 and 32 (average, 22.9), respectively. Failure to detect some samples by conventional PCR may therefore be explained by lower sensitivity compared to the TaqMan mPCR, which also could be related to the fact that the conventional mPCRs were performed with more multiplexing than the TaqMan mPCRs, which has been shown to compromise the sensitivity (Brownie et al., 1997; Markoulatos et al., 2002).
Different template volumes and the addition of BSA were tested in PCR analyses on DNA from each extraction systems, and it was found that optimal template volume varied among the systems (see Materials and Methods), while all systems performed with higher efficiency when BSA was added. BSA or other PCR enhancers have previously been shown to have a beneficial effect on PCR amplifications when the template is extracted from complex clinical samples like feces (Abu Al-Soud and Radstrom, 1998; 2000; Kreader, 1996; Oikarinen et al., 2009; Wilson, 1997), which is known to harbor several inhibitory substances as, for example, dietary components like complex polysaccharides (Monteiro et al., 1997) and human metabolic vast products like bile salts (Lantz et al., 1997) and heme (Akane et al., 1994). Therefore, internal inhibition control is an important issue when dealing with PCR diagnostics of stool samples and may be constructed as a co-amplification of a nondiagnostic locus already present in the sample (16S rDNA in the conventional mPCR used in this study) or as foreign DNA added to the sample before the extraction (PhIV in the TaqMan PCR used in the present study). The same sample that was not identified correctly by the EasyMag Specific A protocol combined with TaqMan mPCR had an inhibitory effect on the internal amplification control, probably due to suboptimal DNA extraction. No other sample impaired the amplification of the internal PCR controls of either the conventional or the TaqMan mPCR assays. Therefore, we conclude that the systems perform well with respect to removal of PCR inhibitors.

A number of samples were PCR-positive for pathogens not detected by the initial culture-based method. These included 2 samples, positive for EIEC by culture, which were also found to contain enterotoxigenic E. coli virulence genes (est or both est and elt) by PCR and 2 samples, culture positive for EPEC, which contained Clostridium difficile virulence genes in the PCR analyses. These PCRs showed the same results when retested, but no analyses were performed to confirm these by culture or PCR targeting alternative loci. Pathogens not detected by culture may be explained by failure to grow or not to be selected as a morphologically distinct colony on the plates. However, because no confirmatory assays were applied here, we cannot exclude the possibility of unspecific amplification.

Except the EasyMag, for which we had the opportunity to test a modified protocol particularly optimized for stool specimens, no additional optimizations were conducted for any of the other extraction systems. It is therefore important to stress out that the performances observed in this study may have come out differently if a careful optimization had been performed on each extraction system. But this was not the objective of the present study. Despite this we believe that the present study brings important data on DNA extraction efficiencies among the commercial extractions systems covering a number of different technologies and shows that an optimized extraction protocol for stool samples combined with TaqMan PCR is a feasible detection method for this type of analysis.

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References


