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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.

Keywords: Commercial kits; DNA extraction; DNA purification; Gastrointestinal pathogens; Stool

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 loose 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.,

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1999; Whitehouse and Hottel, 2007; Yu and Morrison, 2004) and a number of manual and automated commercial methods have been compiled in a couple of reviews (Barken et al., 2007; Espy et al., 2006). Contemporary, commercial extraction systems are for the most part based on chemical and sometimes mechanical lysis in the presence of buffers and chelators to protect liberated DNA from degradation, followed by charge-dependent binding to an immobilized matrix that allows washing and elution of clean DNA. Such methods are designed with ease and functionality in mind, involve few harmful reagents, and some are developed to run on automatic robot equipment, reducing laborious workload when many samples are to be handled in routine diagnostic laboratories. The present study aims to investigate 5 different commercial DNA purification systems for their DNA extraction efficiency on stool samples. Each system was applied on a stool sample collection counting 81 culture-positive samples and the purified template DNA was analyzed by PCR to evaluate the extraction efficiency.

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 Na_2HPO_4, 10 mmol/L KH_2PO_4, 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 I'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./EIEC (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,