Application Note

Detection of virulence factors associated with gastroenteritis

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The QIAxcel® system is highly suitable for PCR-based identification in biomedical research of virulence genes characteristic for diarrheagenic strains of Escherichia coli and Shigella spp.

Introduction

Bacterial gastroenteritis is a predominant cause of morbidity and mortality worldwide, particularly among infants and young children. Causative bacterial pathogens include *Shigella* spp., *Salmonella, Campylobacter jejuni, C. coli, Yersinia enterocolitica,* and diarrheagenic strains of *Escherichia coli* (DEC). Identification of DEC requires differentiating these strains from non-pathogenic members constituting normal intestinal flora. DEC strains can be grouped into 4 main pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (also called Shiga toxin-producing *E. coli* or STEC), and enteroinvasive *E. coli* (EIEC). In addition, enteroaggregative *E. coli* (EAEC) is also speculated to be a causative agent. Phenotypic assays are often used to identify DEC, but the sensitivity and specificity of these methods are not sufficient to differentiate between all pathotypes. Molecular identification and classification of these pathotypes is based on the presence of different chromosomal or plasmid-encoded virulence genes, which are absent in the non-pathogenic, symbiotic *E. coli* (Table 1).

Table 1. DEC pathotypes and virulence genes

Pathotype	Virulence gene	Encoded virulence factor	Amplified fragment (bp)
STEC	Verocytotoxin 1, *stx1* Verocytotoxin 2, *stx2* Intimin, *eaeA*	ST1 ST2 Intimin	370 283 482
EPEC	Intimin, *eaeA*	Intimin	482
ETEC	Heat-labile enterotoxin, *elt* Heat-stable enterotoxin, *esth*	LT ST	322 205
EIEC	Invasive plasmid antigen H, *ipaH*	Invasive plasmid antigen H	619
EAEC*	–	–	–

* Not included in this study.

Materials and methods

DNA extraction and multiplex PCR

Bacterial colonies isolated from fecal samples were suspended in nuclease-free water and boiled for 15 minutes. Lysates were centrifuged for 1 minute at 2000 x *g* and the supernatants were used in amplification reactions. Multiplex PCR was prepared according to a modification of (1) using primers for the genes listed in Table 1 as well as for the 16S ribosomal DNA. Primers were chosen to hybridize to conserved regions of the virulence genes as our main focus is the detection of virulence genes rather than differentiation between gene variants. Amplification of the 16S ribosomal DNA gene within the same reaction was used as an internal control to verify successful extraction of genomic DNA and to exclude the presence of residual PCR inhibitors in cases where no amplification from virulence genes could be detected. Control strains were used as positive controls for all target genes, and a negative control was included in each run to confirm functionality of the multiplex assay.

Figure 1. The QIAxcel system.

Amplification was performed on a Veriti® 96-well Thermal Cycler (Applied Biosystems) with an initial denaturation step at 95°C for 15 minutes, followed by 30 amplification cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 90 seconds, and elongation at 72°C for 90 seconds. A final elongation step at 72°C was performed for 10 minutes. Sample analysis was performed immediately using either conventional agarose gels or the QIAxcel system.

Analysis using agarose gel electrophoresis

Multiplex reactions were analyzed by electrophoresis on 3.5% agarose gels using NuSieve® 3:1 Agarose (Cambrex). A 100 bp ladder was included for estimating fragment size. Gels were run at 130 V for 80 minutes. After electrophoresis, fragments were stained using GelStar® Nucleic Acid Gel Stain (Lonza Bioscience), visualized with UV light, and photographed using a BioDoc-It® Imaging System (UMP, LCC).

Analysis using the QIAxcel system

Multiplex reactions were analyzed on the QIAxcel system using the QIAxcel DNA High Resolution Kit and the OM500 method. The QX Alignment Marker 15 bp/1 kb and QX DNA Size Marker 50–800 bp were used to determine fragment size. Data were visualized using the BioCalculator Software (version 3.5).

Results and discussion

A combination of multiplex PCR and capillary electrophoresis was used in this study for the detection of 6 target genes to differentiate the 4 major DEC pathotypes. Detection of amplified fragments was performed using the QIAxcel system (Figure 1). The QIAxcel system is a capillary electrophoresis instrument that processes samples in batches of 12 and enables analysis of up to 96 samples without manual intervention. The system displays data as both a gel-like image and electropherogram and allows automatic fragment size determination and data storage. No differences were found in the fragment sizes and patterns detected with traditional agarose gel electrophoresis and with the QIAxcel system (Figure 2). As with EIEC, all 4 *Shigella* spp. tested contain the *ipaH* gene, encoding the invasive plasmid antigen H. Therefore, we also successfully identified *Shigella* spp. in this assay. While both technologies allowed us to visualize all amplified fragments, using the QIAxcel system proved to be clearly advantageous in terms of upfront manual preparation time and time of analysis.

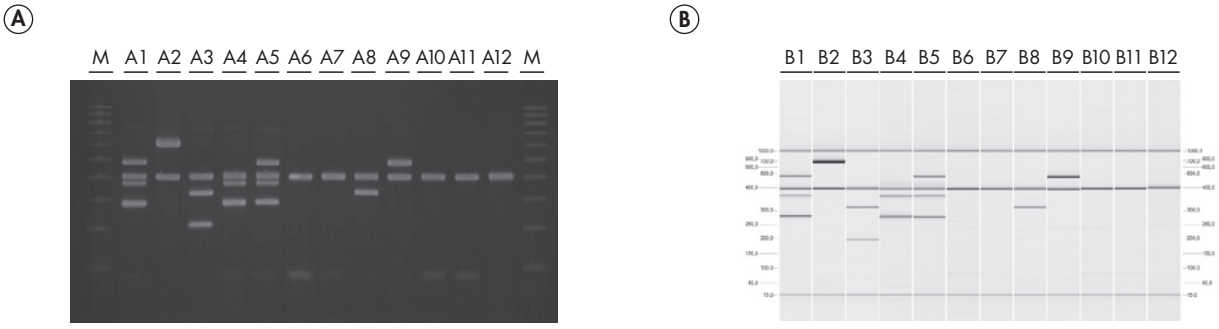

Figure 2. Differentiation of DEC pathotypes and *Shigella* spp. in multiplex PCR. DNA from bacteria isolates was purified, amplified in multiplex PCR, and analyzed using **A.** traditional agarose gel electrophoresis or **B.** the QIAxcel DNA High Resolution Kit on the QIAxcel system. Description of lanes and results are presented in Tables 2 and 3, respectively.
M: 100 bp ladder.

Table 2. Agarose gel electrophoresis analysis (Figure 2A)

Lane	Description	*esth*	*stx2*	*elt*	*stx1*	*16S*	*eaeA*	*ipaH*
A1	Positive control		+		+	+	+	
A2	Positive control					+		+
A3	Positive control	+		+		+		
A4	Sample 1		+		+	+		
A5	Sample 2		+		+	+	+	
A6	Sample 3					+		
A7	Sample 4					+		
A8	Sample 5			+		+		
A9	Sample 6					+	+	
A10	Sample 7					+		
A11	Sample 8					+		
A12	Negative control					+		

Detection of bands of expected size is indicated with "+".

Table 3. QIAxcel analysis of gel image (Figure 2B)

Lane	Description	*esth*	*stx2*	*elt*	*stx1*	*16S*	*eaeA*	*ipaH*
B1	Positive control		+		+	+	+	
B2	Positive control					+		+
B3	Positive control	+		+		+		
B4	Sample 1		+		+	+		
B5	Sample 2		+		+	+	+	
B6	Sample 3					+		
B7	Sample 4					+		
B8	Sample 5			+		+		
B9	Sample 6					+	+	
B10	Sample 7					+		
B11	Sample 8					+		
B12	Negative control					+		

Detection of bands of expected size is indicated with "+".

Conclusions

- Together with the described multiplex PCR assay, the QIAxcel system allowed reliable identification in biomedical research of the 4 main DEC pathotypes as well as *Shigella* spp. in samples where DEC is suspected to be the cause of gastroenteritis.
- By using the QIAxcel system, the workflow in our departments has been streamlined and working conditions for laboratory personnel have been improved by reducing manual pipetting steps to a minimum, by eliminating time consuming gel preparation as well as staining and destaining steps, and by obtaining results in an easy-access digital format.

References

1. Brandal, L.T., Lindstedt, B., Aas, L., Stavnes, T., Lassen, J., and Kapperud, G. (2007) Octaplex PCR and fluorescence-based capillary electrophoresis for identification of human diarrheagenic *Escherichia coli* and *Shigella* spp. J. Microbiological Methods **68:** 331.

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