Application Note

QIAxcel® System — Clostridium difficile ribotype determination

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Abstract

In this application note, we describe the transfer of methods based on agarose gel electrophoresis for ribotype detection and genotype characterization of *Clostridium difficile* to the QIAxcel system. Using the QIAxcel system, we were able to detect the fragment pattern characteristic of the 027 ribotype *C. difficile* strain in both gel and electropherogram views. The system also proved highly suitable for PCR-based detection of the *tcdA* enterotoxin and *tcdB* cytotoxin genes as well as the *tcdC* deletion. These loci together with the *tcdR* and *tcdE* genes form a chromosomal pathogenicity locus (PaLoc) (1, 2). The *cdtA/B* binary toxin gene was also detected.

Introduction

Clostridium difficile is the major cause of nosocomial diarrhoea and antimicrobial-associated colitis. Millions of infections a year cause diarrhoea, sometimes with abdominal pain and vomiting. Enteritis caused by *C. difficile* infection has become an increasing problem in the past few years. Since 2002, severe outbreaks have been reported with increased mortality for both elderly and young patients.

Antibiotics, especially those with a broad-spectrum activity, cause disruption of normal intestinal flora and can lead to overgrowth of *C. difficile*, which flourishes under these conditions. It is suspected that use of these antibiotics drives the formation of hypervirulent strains. Identified hypervirulent strains are of the 027 ribotype and express 3 toxins: enterotoxin TcdA, cytotoxin TcdB, and binary toxin CdtA/B. Furthermore, a deletion within the regulatory *tcdC* gene, normally restricting production of the TcdA and TcdB toxins, is characteristic for these strains.

We evaluated use of the QIAxcel, a capillary electrophoresis system, for ribotyping *C. difficile* strains and analysis of toxin genes. In comparison to conventional agarose gel based methods, this system provides significantly shorter run times and fewer manual handling steps, freeing up time for more demanding lab work and reducing manual error rates.

Materials and methods

Nucleic acid purification and PCR ribotyping

C. difficile isolates were subcultured on CCF (cycloserin, cephalosporin, fructose) selective plates and incubated anaerobically for 24–48 hours. After harvesting, genomic DNA was isolated using a Chelex 100-based method (3) and PCR ribotyping was performed as described previously (4). After PCR amplification, samples were concentrated by heating at 75°C for 55 minutes, the volume was adjusted to 10 µl with QX DNA Dilution Buffer, and analysis was performed on the QIAxcel system using the "OM500" method and QX Alignment Marker 15 bp/1 kb.

PaLoc analysis

The 027 sample, which is positive for ribotyping, was used for detection of the *tcdA, tcdB*, and *cdtA/B* genes and the *tcdC* deletion. Gene sequences were amplified using primers which are described elsewhere (1, 5, 6) and amplicons were analyzed on the QIAxcel system using the "0M500" method and QX Alignment Marker 15 bp/1 kb. DNA izing was performed using the QX DNA Size Marker 50 bp/800 bp.

Results and discussion

C. difficile PCR ribotyping patterns are based on size variations in the 16S–23S intergenic spacer regions of the bacterial rRNA (*rrn*) operon. Traditionally, analysis of these variations is performed using agarose gel electrophoresis. While this analysis method is easy to use and relatively cheap, it also requires long run times as well as significant manual effort to pour and prepare gels and often provides poor resolution. Recently, the use of methods based on capillary electrophoresis has been described to discriminate between different *C. difficile* strains and to analyze infection clusters (7–9).

The QIAxcel system is a capillary electrophoresis system that processes sample in batches of 12 and allows analysis of up to 96 samples without manual intervention. The system displays data as both a gel-like image and electropherogram. The QIAxcel system was used for analysis of *C. difficile* reference strains, and the typical ribotype patterns can be observed in the gel view (Figure 1A and 1B). Comparison of the 027 sample with classical agarose gel electrophoresis reveals a comparable fragment pattern (Figure 1C).

The QIAxcel system also proved to be highly suitable for PCR-based detection of the *tcdA, tcdB*, and *cdtA/B* genes (Figure 2A). The 18 bp deletion of the *tcdC* gene was accurately detected by the QIAxcel BioCalculator Software (Figure 2B).

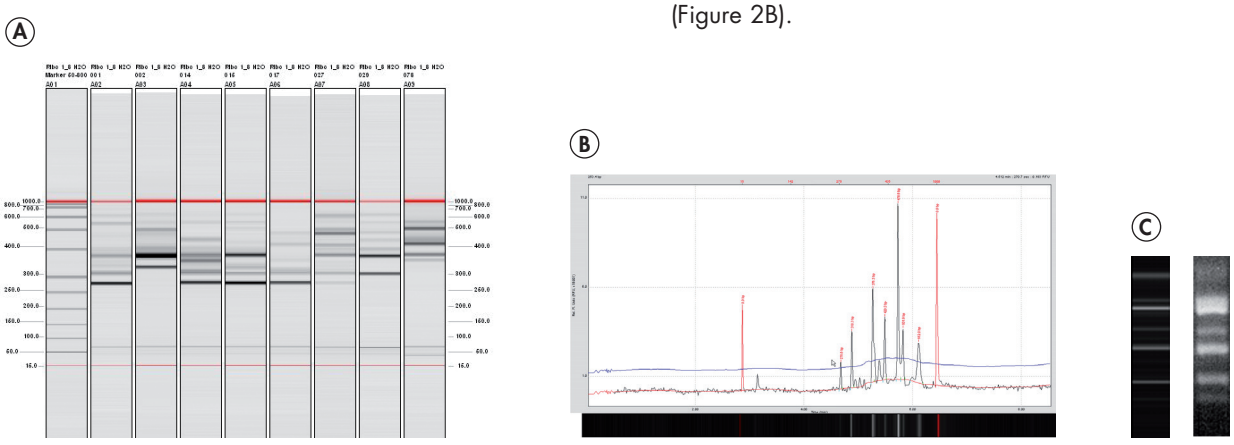

Figure 1. *C. difficile* ribotyping. A. Gel and **B.** electropherogram views of the 027 ribotype pattern obtained with the QIAxcel system. Alignment markers are indicated in red. **C.** Comparison of the 027 ribotype pattern obtained with the QIAxcel method (left) and with the traditional agarose gel method (right).

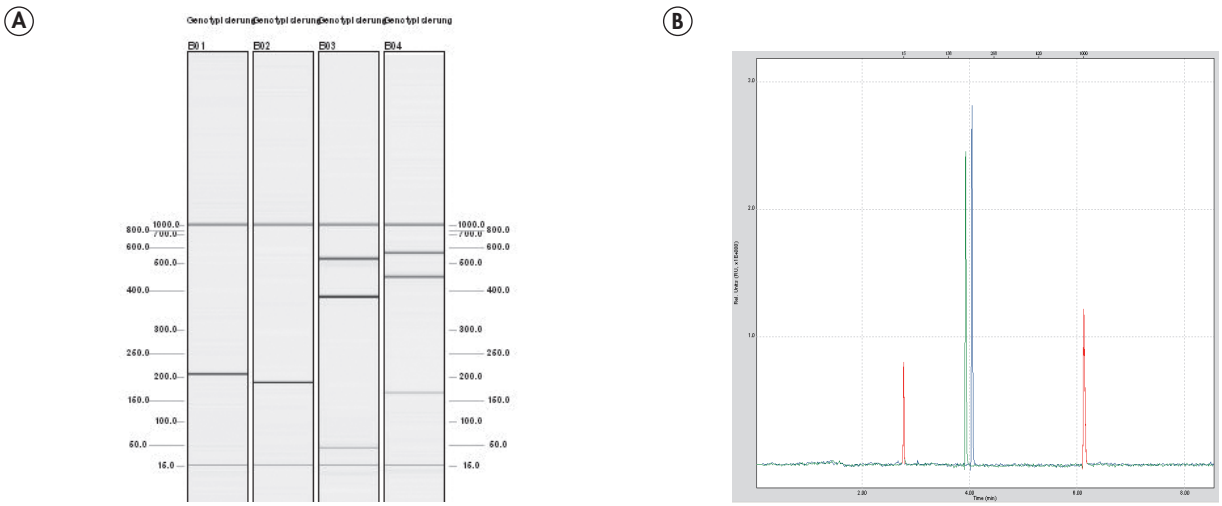

Figure 2. *C. difficile* genotyping. A. Partial genotyping of a *C. difficile* 027 ribotype sample. **B01**: *tcdC* control; **B02**: *tcdC* with deletion; **B03**: *cdtA/B*; **B04**: *tcdA/B*. **B.** Overlay of electropherogram views of B01 (206.8 bp, blue) and B02 (188.4 bp, green) lanes from part **C.** demonstrating the 18 bp *tcdC* deletion. Peaks for the upper and lower alignment marker are indicated in red.

Conclusions

The QIAxcel system proved to be suitable for *C. difficile* ribotyping applications and toxin-gene detection. Due to significantly shorter run times in comparison to conventional methods, the system has the potential to reduce the cost of PCR ribotyping by drastically reducing the hands-on time. Standardized automated processing facilitates inter-laboratory data exchange without the need for cumbersome standardization of equipment, reagents, and operating procedures.

References

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Ordering Information

Product	Contents	Cat. no.
QIAxcel System	Capillary electrophoresis device, including computer, and BioCalculator Analysis software; 1-year warranty on parts and labor	9001421
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNALarge Fragment Kit (600)	QIAxcel DNA Large Fragment Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929006
QIAxcel RNA Quality Control Kit (1200)	QIAxcel RNA Quality Control Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX Alignment Marker, 12-Tube Strips	929102

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