

High Throughput *Clostridium difficile* DNA Isolation

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Introduction

Clostridium difficile bacteria are the major cause of nosocomial antibiotic-associated diarrhea and pseudo-membranous colitis. The expansion of pathogenic strains in hospitals and health care centers must be avoided and outbreak situations must be detected rapidly to prevent increasing costs in patient management. The development of a nucleic acid based testing has become important, because the enzyme linked tests led to false negative results.

Most of the pathogenic *Clostridium difficile* strains produce two well-characterized toxins: toxin A an enterotoxin and toxin B a cytotoxin. Both cause inflammation and damage of colonic mucosa and are recognized as the main virulence factors. Toxin genes (*tdcA*, *tdcB*) are located with three more genes (*tdcC*, *tdcD*, *tdcE*) on the pathogenicity locus which is only present in pathogenic strains.

In this case, a PCR assay was used which can detect toxin genes *tdcA* and *tdcB*, because these *Clostridium difficile* strains have been described to cause most of the serious diseases. A crucial step in NAT based assays is the nucleic acid isolation. This process must be very fast but efficient, reliable and safe. In order to waste no time, the extracted DNA must be ready to use directly after isolation. In this case a reliable high throughput laboratory diagnostic capability for detection of *Clostridium difficile* was evaluated.

In general bacterial DNA extraction is performed as follows: Stool samples are diluted in 5 ml NaCl (0.9%) and centrifuged for 3 min with 3000 rpm. 200 µl of the supernatant is used for the automated nucleic acid isolation. The automated DNA isolation is performed with the **chemagic Magnetic Separation Module I** (chemagic MSM I) and the **chemagic Viral NA/gDNA Kit special** (art.No. 1049). This chemagen kit facilitates viral and bacterial NA isolation from all body fluids. To realize high throughput we decided for the **chemagic 96 Rod Head** to perform 96 DNA isolation processes parallel in standard 96 well plates within less than 1 hour. The **chemagic MSM I** uses chemagen's patented separation technology, which is based on switchable magnetization and rotation of steel needles to realize efficient mixing and complete separation of the magnetic particles. The DNA is usually eluted in 50 µl elution buffer, provided in the chemagic Kit. The subsequent Real Time PCR (RT-PCR) is performed with the REALStar® *Clostridium difficile* PCR Kit 1.0 (Astra Diagnostics, Germany).

Sensitivity of nucleic acid isolation proven by RT-PCR analysis

Interlab. test No.	Probe	CFU*/ml	PCR chemagic MSM I Toxin A	PCR chemagic MSM I Toxin B
CD09-01	C. diff O27	4600	positive	positive
CD09-02	C. diff O27	460.000	positive	positive
CD09-03	C. diff O27	460	positive	positive
CD09-04**	C. diff O17	80.000	negative	positive
CD09-05	C. diff O27	4600	positive	positive
CD09-06	negative	negative	negative	negative
CD09-07	C. sordellii	210.000	positive	positive
CD09-08**	C. diff O17	800	negative	positive
CD09-09**	C. diff O17	8000	negative	positive
CD09-10	C. diff O27	46.000	positive	positive

Table 1:

Clostridium difficile

spiked samples from a QCMD test panel (2009) were isolated with the chemagic MSM I and the chemagic Viral RNA/gDNA Kit special and subsequently tested via RT-PCR.

*CFU = Colony Forming Unit

**Ribotype O17:

negative for Toxin A but positive for Toxin B

To test whether cross contamination occurs during the isolation process and how sensitive the extraction method is, a QCMD test panel from 2009 was used. The QCMD test panel contained samples with indicated CFU/ml (Table 1) and different ribotypes of ***Clostridium difficile***. Samples CD 09-04, CD 09-08 and CD 09-09 represent Ribotype O17 which is negative for Toxin A but positive for Toxin B. This result emphasizes the requirement for detection of both toxin genes, because this strain causes antibiotic associated diarrhea and colitis as well as strains which have both toxin genes. Testing only for toxin A will lead to false negative results.

Moreover the results given in Table 1 show the efficiency of the DNA isolation and the sensitivity of the subsequent RT-PCR, because sample number CD 09-03 was tested positively, although the CFU value was only 460 CFU/ml. Even if the bacterial DNA concentration is very low, reliable results can be achieved with the **chemagic MSM I**.

We have determined an inhibition rate of less than 5% in our daily routine diagnostics. Thus almost all of the PCR inhibiting factors are separated from the bacterial DNA during the isolation process. Although high positive samples were placed right next to negative samples the RT-PCR revealed no cross contamination. This strongly indicates that the automated DNA isolation is absolutely reliable.

In conclusion, chemagen's high performance automated DNA extraction method leads to high quality nucleic acids, ready to use for the subsequent RT-PCR. This nucleic acid based method could displace the enzyme linked method, because it is more reliable, faster and more sensitive.