Rapid Detection Method of Live *Clostridium difficile*

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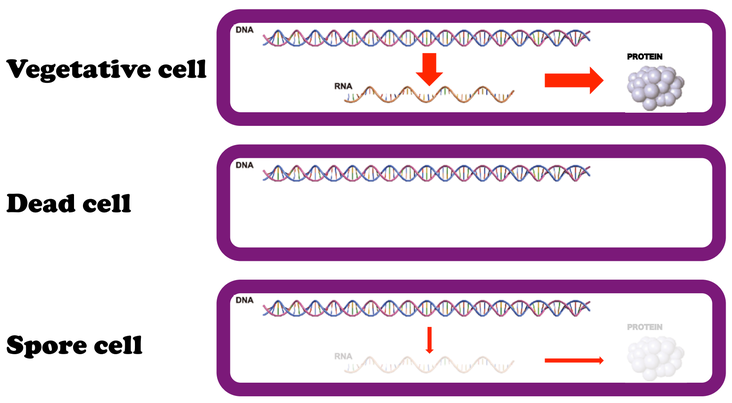
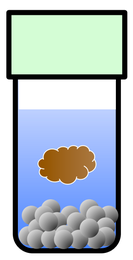
Clostridium difficile **is a principle pathogen causing antibiotic-associated diarrhea and colitis. There are several methods for laboratory diagnosis of *C. difficile* Infection, such as *C. difficile*-culture, enzyme immunoassay (EIA) detecting toxins, and PCR detecting the toxin genes. However, the culture method can not distinguish toxigenic cells from nontoxigenic cells. The detection sensitivity of EIA is low. PCR can not distinguish between live and dead cells. We focused that RNA is transcribed in only live cells. The rapid detection method of vegetative toxigenic *C. difficile* was established by using reverse transcription PCR (RT-PCR).**

Comparison of RNA and DNA in vegetative, dead, and spore cell



	Vegetative cell	Dead cell	Spore cell
RNA	+	-	+ (partial)
DNA	+	+	+

Extraction of RNA



Sample + ISOGEN® + Glass beads → Disruption by shaking → DNase treatment → RNA

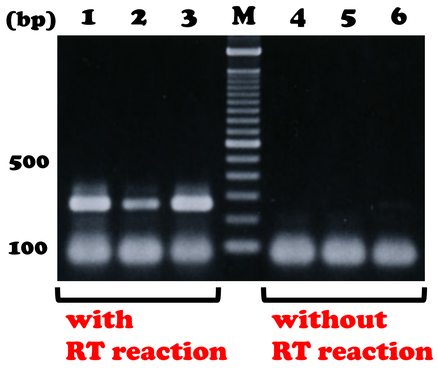
RT-PCR condition

Target gene: *tcdA* non-repeating sequence(Toxin A gene)
Primers: NK3 and NK2 (J. Clin. Micro., 1991, 29, 33-7)

RT condition: 30°C 10 min; 42°C 20 min; 99°C 5 min; 4°C 5 min

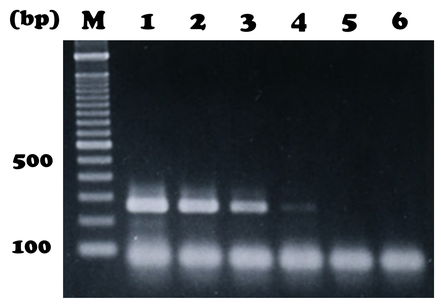
PCR condition: 95°C 20 sec; 55°C 2 min — 35 cycles

RT-PCR of pure culture



1, 4: Strain KO20 ($A^+B^+CDT^-$)
2, 5: Strain TR15 ($A^-B^+CDT^-$)
3, 6: Strain JND06-50 ($A^+B^+CDT^+$)

Detection limit of RT-PCR



Amount of RNA (strain KO20)
1: 200 pg
2: 20 pg
3: 2 pg
4: 0.2 pg
5: 20 fg
6: 2 fg

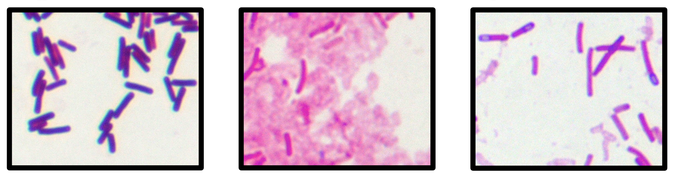
Spike test <Preparation of RNA>

C. difficile strain KO20 was cultivated for 16 hours at 35°C in anaerobic chamber. OD600 was adjusted to 0.5 by media which was used for cultivation. The culture of 0.1 ml (approx. 10^6 CFU) was ...

... used as vegetative cell. → Gram stain of vegetative cell

... autoclaved at 121°C for 15 min. → Gram stain of dead cell

... soaked in 70% ethanol for 16 hours. → Gram stain of spore cell



\+ Stool specimen (*C. difficile* negative)

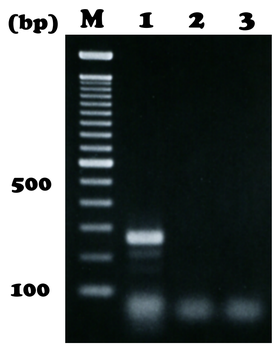
1. + ISOGEN® + Glass beads
2. Disruption by shaking
3. DNase treatment

RNA extracted from human stool which contained vegetative cell of *C. difficile* strain KO20

RNA extracted from human stool which contained dead cell of *C. difficile* strain KO20

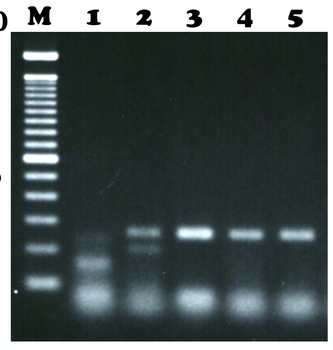
RNA extracted from human stool which contained spore cell of *C. difficile* strain KO20

Spike test <Result of RT-PCR>



1: RNA extracted from human stool which contained vegetative cell of *C. difficile* strain KO20
2: RNA extracted from human stool which contained dead cell of *C. difficile* strain KO20
3: RNA extracted from human stool which contained spore cell of *C. difficile* strain KO20

Stool specimen <Result of RT-PCR>



1: Specimen 1
Culture method (-)
C. DIFF QUIK CHEK COMPLETE® (-)
2: Specimen 2
Culture method (+); $A^+B^+CDT^-$
C. DIFF QUIK CHEK COMPLETE® (+)
3: Specimen 3
Culture method (+); $A^+B^+CDT^-$
C. DIFF QUIK CHEK COMPLETE® (+)
4: Specimen 4
Culture method (+); $A^-B^+CDT^-$
C. DIFF QUIK CHEK COMPLETE® (+)
5: Specimen 5
Culture method (+); $A^+B^+CDT^-$
C. DIFF QUIK CHEK COMPLETE® (+)

Conclusion

The toxigenic *C. difficile* $A^+B^+CDT^-$, $A^-B^+CDT^-$, and $A^+B^+CDT^+$ were detected by this method.

The vegetative toxigenic *C. difficile* in stool specimen was detected by this method.

This method detected only vegetative cell, but not dead and spore cell.

This method completed within 5 hours.

These results suggest that this method is valuable as laboratory test.