

## Clostridium Difficile Detection Protocol

### A. Introduction

This protocol uses end point PCR<sup>†</sup> with TaqMan™ probes to test for *Clostridium difficile* (*C. difficile*). The target sequence is the toxin B (*tcdB*) gene, which is specific to toxigenic *C. difficile*.

PCR run time is 29 min.

### B. Equipment

- Spartan DX™ instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

### C. Materials

- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- BIOTUB-QT kit (Biotools, Cat. No. 90.572C)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers that recognize the *tcdB* gene (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) that recognizes the *tcdB* gene (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>tcdB</i> primers	Tgg ATT TgT gAC TgT Agg CgA Tg	CAC CTg TTT gTA ACA CTC CAC TTT gg	131
<i>tcdB</i> probe	6-FAM - Tgg Tgg AgC TgC TTC AAT Tgg AgA gA - BHQ-1		

6FAM = 6-carboxy-fluorescein, BHQ-1 = Black Hole Quencher 1

**Table 1.** Primer/probe sequences and amplicon size.

### D. Preparation

1. Any commercially-available DNA purification kit may be used
2. Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
3. Set up thermal cycling program as per Table 2

Step	Temperature	Time	Cycles
Initial denaturation	95.0°C	30 s	1
Denaturation	95.0°C	18 s	45
Annealing/extension	50.0°C	20 s	45

**Table 2.** Cycling parameters.

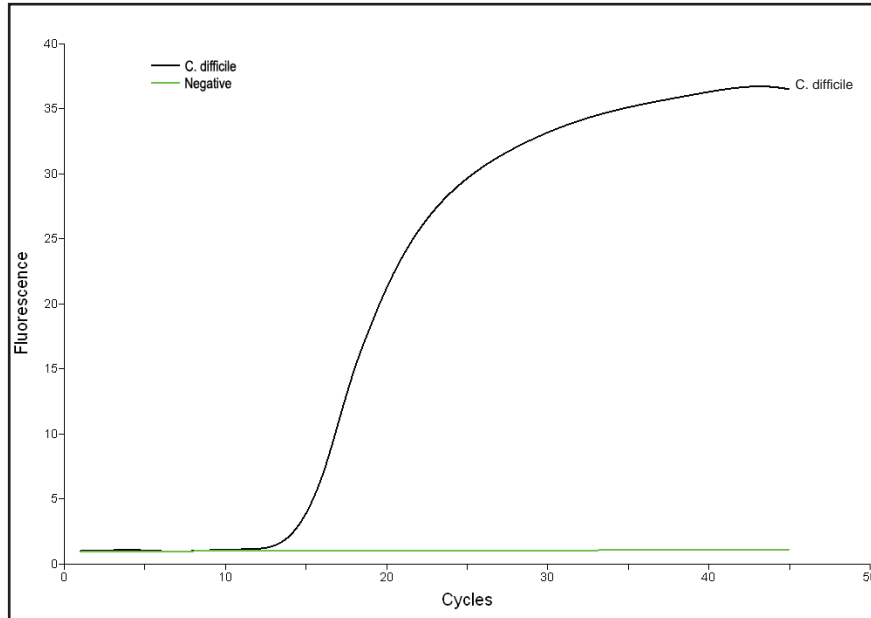
## E. Protocol

1. Prepare a master mix for *tcdB* as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
  - Tubes 1: 17.5 µl of *tcdB* master mix
  - Tube 2: 17.5 µl of *tcdB* master mix
  - Tube 3: 17.5 µl of *tcdB* master mix
3. In a separate lab area, add the following to each tube:
  - Tube 1: 2.5 µl of purified DNA (sample in question)
  - Tube 2: 2.5 µl of known *tcdB*-positive DNA (positive control)
  - Tube 3: 2.5 µl of sterile water (negative control)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert samples into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater than 5
9. Example of real-time graph is depicted in Figure 1

Reagent	<i>tcdB</i> Master Mix	
	Reaction Formulation	Volume
QUANTIPROBES Reaction Mix (2X)	10 µl x (____+0.5*) Sample #	
Forward primer (10 µM)	1 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	1 µl x (____+0.5*) Sample #	
Probe (1 µM)	2 µl x (____+0.5*) Sample #	
Sterile water	3.5 µl x (____+0.5*) Sample #	
<b>Total volume of master mix</b>	<b>17.5 µl/reaction</b>	<b>____ µl</b>

\* Recommended volume correction factor for pipetting error.

**Table 3.** Components of PCR master mix for *tcdB*.



**Figure 1.** Real-time PCR result for toxigenic *Clostridium difficile* (*C.difficile*).

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.

#### Disclaimer

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