

**Supplementary Table S1.** Primer and probe sequences used for ddPCR and RT-qPCR

Primer name	Sequence (5' to 3')
<i>tcdB-f</i>	ATATCAGAGACTGATGAG
<i>tcdB-r</i>	TAGCATATTCAGAGAATATTGT
<i>tcdB-p</i>	FAM-CTGGAGAATCTATATTTGTAGAAACTG-BHQ

**Supplementary Table S2.** PCR oligonucleotides for *C. difficile tcdB* gene

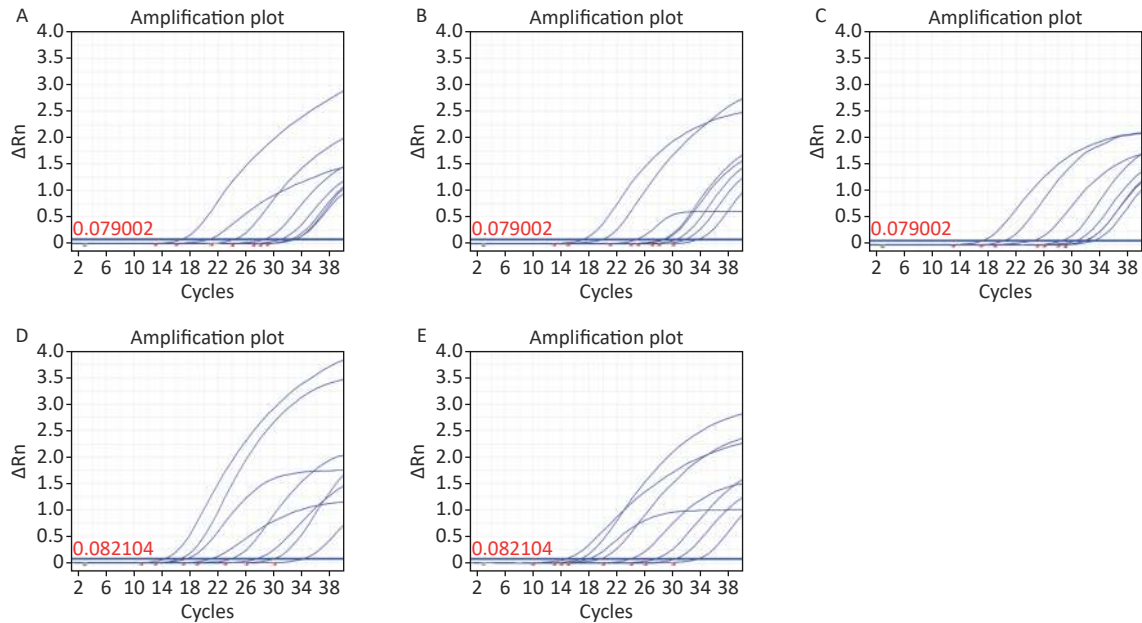
Primer name	Primer code sequence	Sequence (5' to 3')
Primer1	<i>tcdB-F3</i>	CCAAAGTGGAGTGTACAAACAGGTG
	<i>tcdB-R3</i>	GCATTTCTCCATTCTCAGCAAAGTA

**Supplementary Table S3.** Plasmid sequences for ddPCR and RT-qPCR amplification

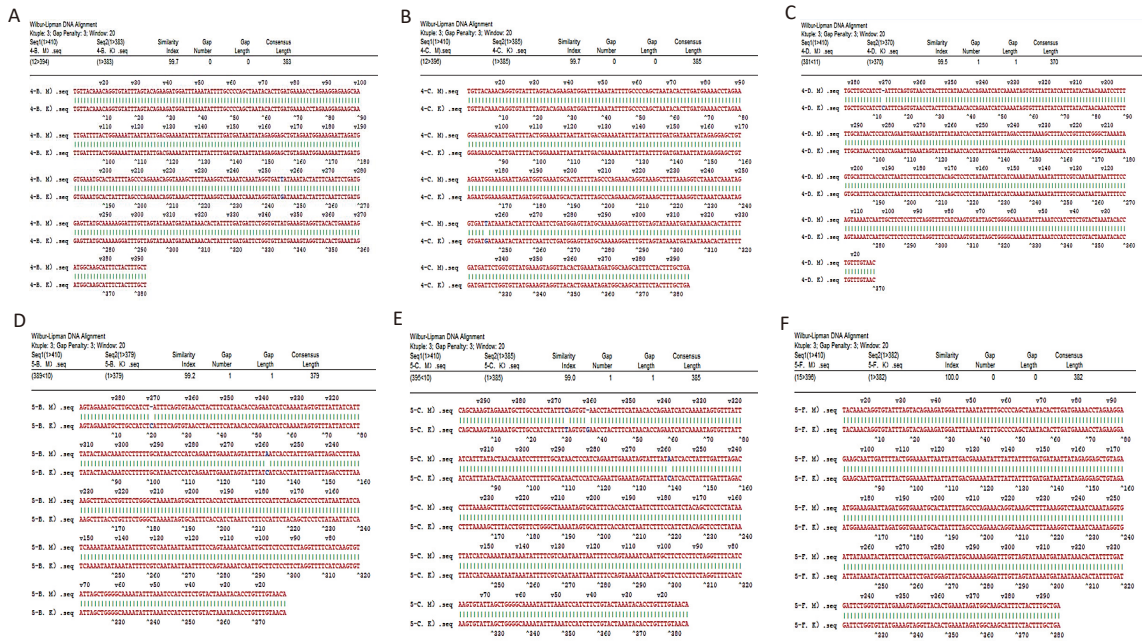
Name	Sequence (5' to 3')
Suquence 1 (103 bp)	ATATCAGAGACTGATGAGGGATTTAGTATAAGATTTATTAATAAAGAAACTGGAGAA TCTATATTTGTAGAAACTGAAAAACAATATTCTCTGAATATGCTA

**Supplementary Table S4.** Test results of clinical isolates

Clinical isolate	Method		
	ddPCR	Sanger	RT-qPCR
106	+	+	+
11151	+	+	+
112813	+	+	+
1904084	+	+	+
D4	+	+	+
D5	+	+	+
1904082	-	-	-



Supplementary Figure S1. Annealing temperature optimization. (A) 52 °C; (B) 52 °C; (C) 52 °C; (D) 52 °C; (E) 52 °C.



Supplementary Figure S2. Clinical isolate Sanger. (A) 106; (B) D4; (C) D5; (D) 112813; (E) 1904084; (F) 11151.