

Chlamydia Trachomatis Detection Protocol

A. Introduction

This protocol uses end point PCR[†] with BHQ^{plus}[™] probes to test for *Chlamydia trachomatis* (CT). The target sequence in this assay is the major outer membrane protein (*MOMP*) gene, which is specific to CT.

PCR run time is 37 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)
- QUANTIPROBES Reaction Mix (Biotools, Cat. No. 10.601)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers which recognize the *MOMP* gene (Integrated DNA Technologies)(Table 1)
- BHQ^{plus} probe (Biosearch Technologies) for the *MOMP* gene (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>MOMP</i> primers	ggg gAA TCC TgC TgA ACC AA	TCA CAC CAA gTg gTg CAA ggA	93
<i>MOMP</i> probe	6FAM- Tgg AgA TCC TTg CgA TCC -BHQ ^{plus}		

6FAM = 6-carboxy-fluorescein, BHQ^{plus} = Black Hole Quencher *plus*

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	32 s	1
Denaturation	95.0°C	28 s	50
Annealing/extension	44.0°C	20 s	50

Table 2. Cycling parameters.

E. Protocol

1. Prepare a master mix for *MOMP*, as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
 - Tube 1: 17 µl of *MOMP* master mix
 - Tube 2: 17 µl of *MOMP* master mix
 - Tube 3: 17 µl of *MOMP* master mix
 - Tube 4: 17 µl of *MOMP* master mix
3. In a separate lab area, add the following to each tube:
 - Tube 1: 1.5 µl of purified DNA (sample in question) + 1.5 µl of sterile water
 - Tube 2: 1.5 µl of known *C. trachomatis*-positive DNA + 1.5 µl of sterile water (positive control)
 - Tube 3: 1.5 µl of purified DNA (sample in question) + 1.5 µl of sterile water (spiked control)
 - Tube 4: 3 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	MOMP Master Mix	
	Reaction Formulation	Volume
QUANTIPROBES Reaction Mix (2X)	10 µl x (____+0.5*) Sample #	
Forward primer (10 µM)	2 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	2 µl x (____+0.5*) Sample #	
Probe (1 µM)	2 µl x (____+0.5*) Sample #	
Total volume of master mix	17 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix

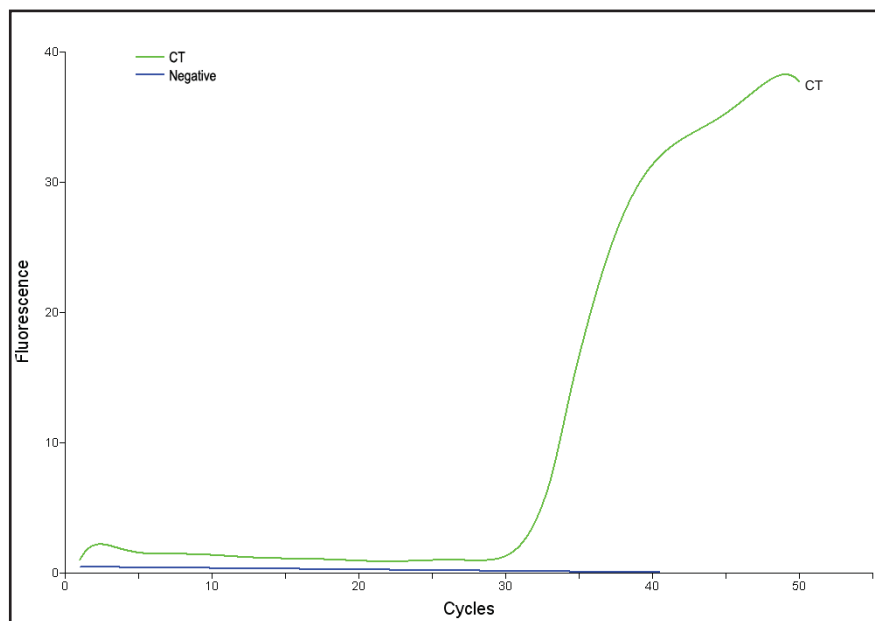


Figure 1. Real-time PCR result for *C. trachomatis*.

† - 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™.

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