

## Neisseria Gonorrhoeae Detection Protocol

### A. Introduction

This protocol uses end point PCR<sup>†</sup> with TaqMan<sup>®</sup> probes to test for *Neisseria gonorrhoeae* (NG). The target sequence in this assay is the *porA* pseudogene which is specific to *N. gonorrhoeae* and not present in other *Neisseria* species.

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 *porA* pseudogene (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) for the *porA* pseudogene (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>porA</i> primers	TTg gCg gCT CAg TTg gAT TT	ATC gAC ACC ggC gAT gAT TT	155
<i>porA</i> TaqMan probe	6FAM- CCC gCg CAT CAg CTA TgC CCA -BHQ1		

6FAM = 6-carboxy-fluorescein, BHQ1 = 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

### E. Protocol

1. Prepare a master mix for *porA*, as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
  - Tube 1: 16 µl of *porA* master mix
  - Tube 2: 16 µl of *porA* master mix
  - Tube 3: 16 µl of *porA* master mix
3. In a separate lab area, add the following to each tube:
  - Tube 1: 4 µl of purified DNA (sample in question)
  - Tube 2: 4 µl of known NG-positive DNA (positive control)
  - Tube 3: 4 µl of sterile water (negative control)

4. Mix and spin down reaction tubes
5. Overlay reaction mixtures with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert tubes 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. Representative real-time PCR results are shown in Figure 1

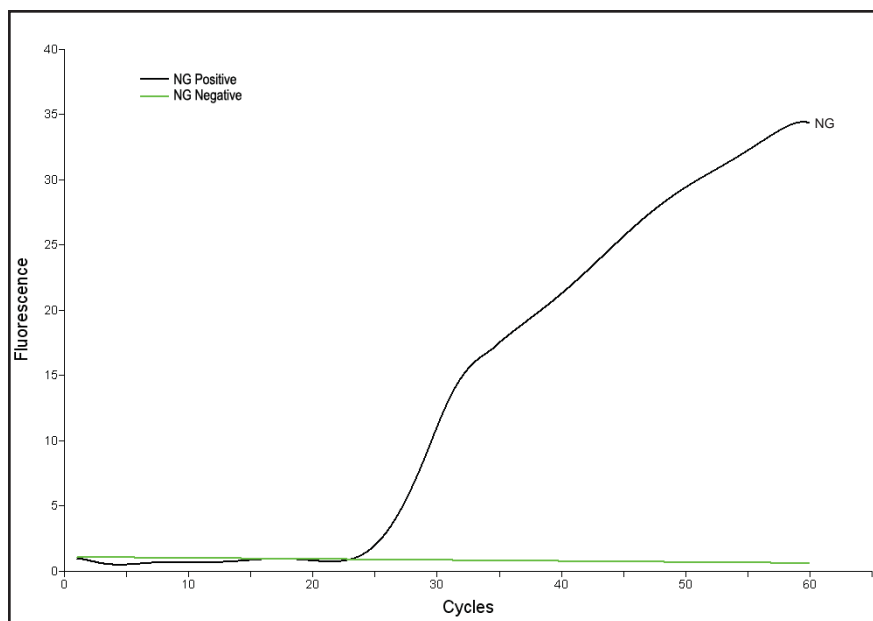
Step	Temperature	Time	Cycles
Initial denaturation	95.0°C	32 s	1
Denaturation	95.0°C	28 s	50
Annealing/extension	44.0°C	20 s	50

**Table 2.** Cycling parameters.

Reagent	porA 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 #	
<b>Total volume of master mix</b>	<b>16 µl/reaction</b>	<b>____ µl</b>

\* Recommended volume correction factor for pipetting error.

**Table 3.** Components of PCR master mix for *N. gonorrhoeae*.



**Figure 1.** Real-time PCR result for *N. gonorrhoeae*.

† - 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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