

Treponema pallidum pallidum (Syphilis) Detection Protocol

A. Introduction

This protocol uses end point PCR[†] with TaqMan[®] probes to test for *Treponema pallidum pallidum* (*T. pallidum pallidum*), the bacterium that causes Syphilis. The target sequence in this assay is the DNA polymerase I gene (*Pol A*) which is specific to *T. pallidum pallidum* and is not present in other *T. pallidum* subspecies.

PCR run-time is 30 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)
- QUANTIMIX EASY PROBES (QUANTIPROBES) kit (Biotools, Cat. No. 10.601)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers that recognize *Pol A* (Integrated DNA Technologies) (Table 1)
- TaqMan probes that recognize *Pol A* (Biosearch Technologies)(Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>Pol A</i> primers	gAg TgC ACA gAA CAg CAT ggg gTA	gCT ggT gCA TgA CaG CTT ggA	76
<i>Pol A</i> probe	6FAM - TgC ATC TgC TgT gCA ggA TCC ggC A-BHQ-1		

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 to extract Syphilis DNA
2. Turn on Spartan DX instrument and leave it to 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°C	10 s	1
Denaturation	95°C	25 s	40
Annealing/extension	50°C	21 s	40

Table 2. Cycling parameters.

E. Protocol

1. Prepare a master mix as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
 - Tube 1: 15 µl of Syphilis master mix
 - Tube 2: 15 µl of Syphilis master mix
 - Tube 3: 15 µl of Syphilis master mix
 - Tube 4: 15 µl of Syphilis 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) + 2.5 µl of sterile water
 - Tube 2: 2.5 µl of known Syphilis-positive DNA + 2.5 µl of sterile water (positive control)
 - Tube 2: 2.5 µl of purified DNA (sample in question) + 2.5 µl of known Syphilis-positive DNA (spiked control)
 - Tube 3: 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	Pol A Master Mix	
	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 (10 µM)	0.5 µl x (____+0.5*) Sample #	
Sterile water	2.5 µl x (____+0.5*) Sample #	
Total volume of master mix	15 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix.

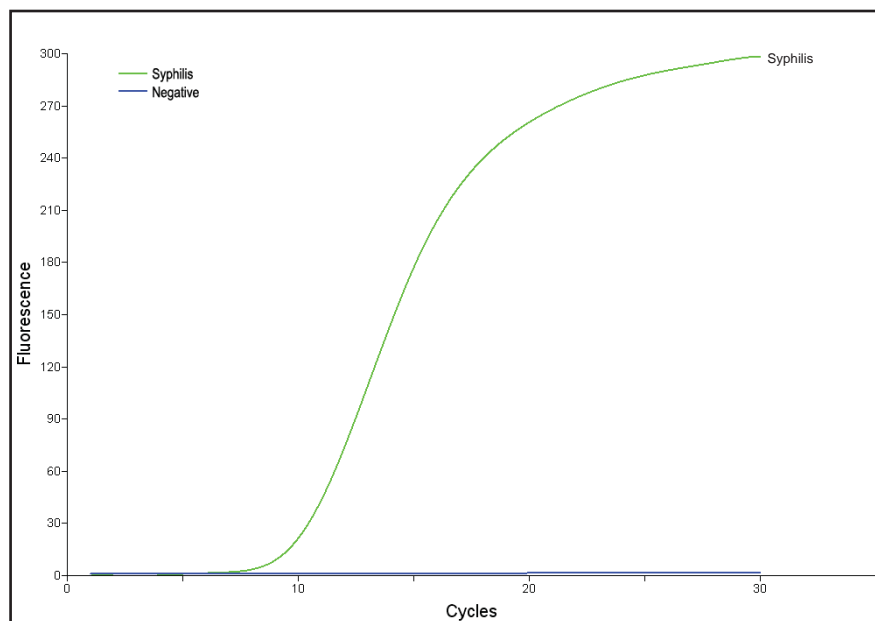


Figure 1. Real-time PCR result for *T. pallidum pallidum* (Syphilis).

† - 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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