Plasmodium Species Detection Protocol

A. Introduction

This protocol uses end point PCR† with TaqMan™ probes to test for *Plasmodium* (malaria) parasites. The target sequence is the *18S ribosomal RNA* (*18S*) gene, with primers that specifically identify *P. ovale, P. vivax, P, malariae*, and *P. falciparum*.

PCR run time is 35 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 *18S* gene (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) that recognizes the *18S* gene (Table 1)

Primer/Probe	Forward (5′-3′)	Reverse (5′-3′)	Amplicon size (bp)
18S primers‡	gTT TAA ggC AAC AAC Agg T	CAA TAA TCT ATC CCC ATC ACG A	178-207 bp
18S probe	6-FAM - ggC TgC ACg CgT gCT ACA CTg - BHQ-1		

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

‡ - Safeukui I, et al. (2008). Evaluation of FRET real-time PCR assay for rapid detection and differentiation of Plasmodium species in returning travellers and migrants. *Malar J.* 7:70

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	22 s	50
Annealing/extension	40.0°C	19 s	50

Table 2. Cycling parameters.

E. Protocol

1. Prepare a master mix for as per Table 3
2. To test 1 unknown sample, we recommend setting up reactions as follows:
   - Tube 1: 16 µl of *18S* master mix
   - Tube 2: 16 µl of *18S* master mix
   - Tube 3: 16 µl of *18S* master mix
   - Tube 4: 16 µl of *18S* master mix
3. In a separate lab area, add the following to each tube:
   - Tube 1: 2 µl of purified DNA (sample in question) + 2 µl of sterile water
   - Tube 2: 2 µl of known *18S*-positive DNA + 2 µl of sterile water (positive control)
   - Tube 3: 2 µl of purified DNA (sample in question) + 2 µl of known *18S*-positive DNA (spiked control)
   - Tube 4: 2 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 that 5
9. Example of real-time graph is depicted in Figure 1

Reagent	*18S* 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)	0.4 µl x (_____+0.5*) Sample #	
Sterile water	3.6 µl x (_____+0.5*) Sample #	
Total volume of master mix	**16 µl/reaction**	**____ µl**

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix for *18S* gene.

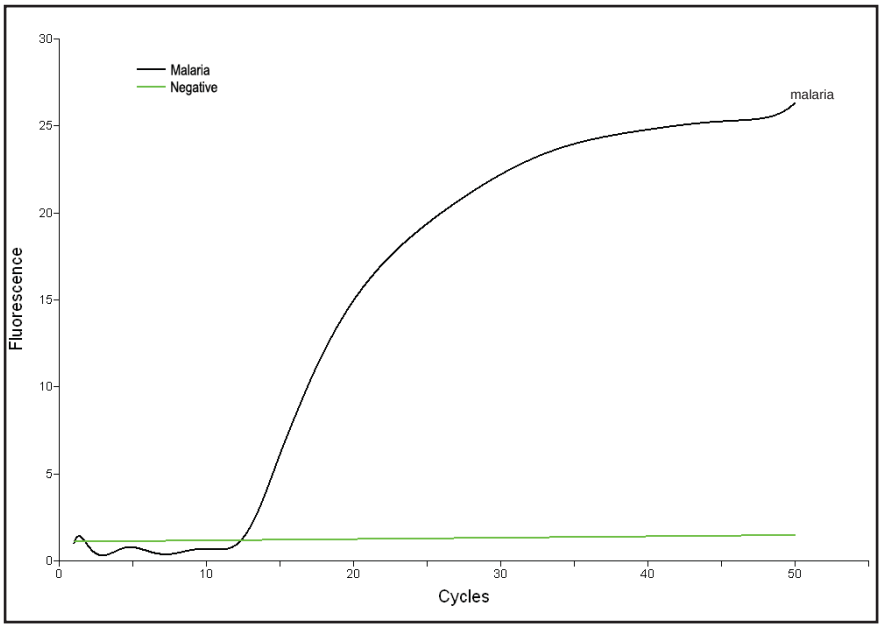

Figure 1. Real-time PCR result for *Plasmodium* (malaria).

† - 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™.