Supplement 1. Vakkila et al. 2014

Assay protocol for *S. pyogenes* specific test applying quantitative polymerase chain reaction

Sample preparation

Throat swab samples stored frozen in RTI sample buffer of the mariPOC® test system (ArcDia International Oy Ltd) were thawed and total DNA was extracted with PrepMan™ Ultra sample preparation reagent (Applied Biosystems, cat 4318930). Fifty (50) µl of each sample was centrifuged at 16 100 g for 10 minutes. Supernatants were discarded and 50 µl of PrepMan™ Ultra sample preparation reagent was added over the pellet. The tubes were vortexed for 30 seconds and placed into a heat block (100 °C) for 10 minutes. The samples were allowed to cool down at room temperature for a few minutes and centrifuged at 16 100 g for 10 minutes. The supernatants were transferred to new tubes and used for PCR.

Real-time quantitative PCR

Real-time detection of the quantitative PCR (qPCR) reaction utilized the fluorometric measurement of the SYBR® Green I dye that binds to double stranded DNA and fluoresces only when bound to DNA. The target gene for the detection of *Streptococcus pyogenes* was DNaseB and the target DNA sequence length was 144 bp. Primers were purchased from Sigma and their sequences are shown in table 1.

Table 1 Sequences of the primers for DNaseB gene

Primer	Sequence	Base positions in DNaseB gene sequence
Forward	5´-ACCCAAAATGTAGGAGGTCGT-3´	556- 576
Reverse	5´-TCTTGGAATCAACTCGTCTGC-3´	679- 699

The qPCR master mix was done by combining KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems, 2X stock, cat KK4610), primers and nuclease free water (Table 2). Real-time

qPCR was performed using 2 µl of extracted DNA sample with a total reaction volume of 10 µl.

Table 2 Reagent volumes in qPCR master mix

Reagent	Volume (Concentration in reaction)
KAPA SYBR FAST qPCR Master Mix (2X)	5 µl (1X)
Forward primer (10 µM)	0.2 µl (0.2 µM)
Reverse primer (10 µM)	0.2 µl (0.2 µM)
Nuclease free water	2.6 µl
Total	8 µl

LightCycler® 480 II (Roche) was used to perform the qPCR. Used PCR temperature parameters are shown in table 3.

Table 3 The program for qPCR run with LightCycler® 480 II

Program	Cycle(s)	Analysis	Temperature	Time	Analysis parameter
Pre-incubation	1	None			
			95 °C	3 min	
Amplification	40	Quantification			
			95 °C	10 sec	
			59 °C	20 sec	
			72 °C	10 sec	Single
Melting curve	1	Melting curve			
			95 °C	30 sec	
			65 °C	1 min	
			97 °C	-	Continuous, 5 pictures/ °C
Cooling	1	None			
			40 °C	30 sec	

The data analysis for each sample included quantification cycle (C_q) and melting curve analysis. A sample was reported to be positive for *Streptococcus pyogenes* when test results were cycle 35 or lower for C_q and when melting temperature was between 81.4 - 84.0 °C.