Real-time PCR detection of *Staphylococcus aureus*

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The Spartan DX™ enables on-demand real-time PCR identification of Staphylococcus aureus in less than 30 min with DNA extracted from bacterial colonies using a rapid boiling lysis method.

Introduction

Staphylococcus aureus is a significant human pathogen that causes illnesses ranging from minor skin infections and abscesses, to life-threatening diseases such as pneumonia, meningitis, endocarditis, Toxic Shock Syndrome (TSS), and septicemia. In the microbiology laboratory, detection of *S. aureus* is performed by culture and biochemical tests. More recently, researchers have designed PCR and real-time PCR assays for *S. aureus* that are more sensitive and specific than traditional methods (Ref 1, 2).

The advantage of real-time PCR is that diagnostic results are available immediately, in real-time, but the disadvantage is that the assays must be performed on batched machines such as the LightCycler® (Roche Diagnostics) or ABI PRISM® 7700 (Applied Biosystems), which are expensive and require extensive technical training and expertise (Ref 3).

The Spartan DX is a real-time PCR device designed for on-demand DNA testing that may be operated by minimally-trained personnel. It accommodates 4 samples at a time, compared to 32, 96, or 384 with batched machines.

The purpose of this study was to determine the performance of the Spartan DX with a real-time PCR assay for *S. aureus*.

Materials and Methods

DNA extraction

Template DNA was prepared from 10 different clinical isolates of *S. aureus* using a rapid boiling lysis protocol. Briefly, individual bacterial colonies were suspended in 100 µl of sterile water and incubated in a screw-cap microcentrifuge tube for 10 min in a boiling water bath. A 2 µl aliquot of the crude lysate was used as the template for real-time PCR.

Real-time PCR

Oligonucleotide primers were designed against the *nuc* gene that encodes a thermostable nuclease specific to *S. aureus*. The forward primer sequence was 5′-gcg att gat ggt gat acg gtt-3′, and the reverse primer sequence was 5′-agc caa gcc ttg acg aac taa agc-3′ (Ref 1). The expected amplicon size was 281 bp. Components of the PCR amplification mixture are listed in Table 1 and cycling parameters are listed in Table 2. Note that a two-temperature cycling program was performed by combining the annealing and extension steps. Reactions were performed in 0.2 ml thin-wall flat cap PCR tubes (Axygen, Cat. No. PCR 02C), and topped with 15 µl of mineral oil (Biotools, Cat. No. 20.032) to prevent evaporation. SYBR Green® I dye (Invitrogen, Cat. No. S-7563) was used for fluorescent detection. To confirm the real-time PCR results, 8 µl of the amplification products were analyzed by agarose gel electrophoresis.

Results

For 30 cycles of real-time PCR, the total run time on the Spartan DX was 26 min. Real-time PCR results with SYBR Green I were positive for all 10 samples. The threshold cycle (Ct) values ranged from cycles 13 to 17. Specific

Component	Final amount
10X PCR Reaction Buffer (No $MgCl_2$) (Invitrogen)	1X
$MgCl_2$ (Invitrogen)	2.5 µM
dNTP mix (Invitrogen)	0.25 µM
Taq DNA polymerase (Invitrogen)	1 U
SYBR Green I (Invitrogen)	0.5 X
PCR primers (Sigma-Aldrich)	1 µM each
Template DNA	2 µl
Sterile water	
Total reaction volume	**20 µl**

Table 1. Components of PCR amplification mixture.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	20 s	30
Annealing/extension	60°C	20 s	30

Table 2. Cycling parameters.

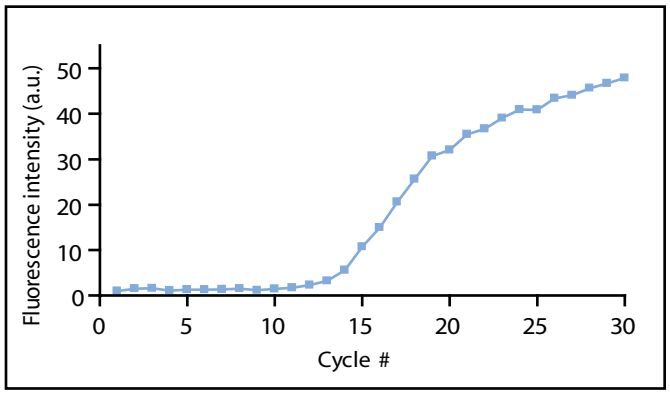

Figure 1. Real-time PCR result with SYBR Green I.

amplification was verified by fluorescence (Figure 1) and gel electrophoresis (Figure 2) for selected samples.

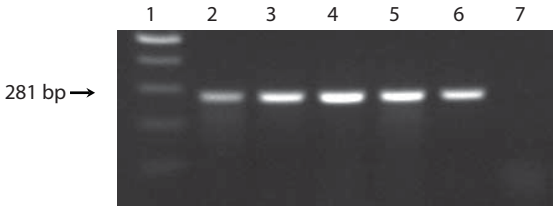

Figure 2. Specific amplification (281 bp) is verified by gel electrophoresis (Lanes 2-6). Lane 1 contains 100 bp DNA Ladder (Invitrogen) and Lane 7 is a negative, no DNA, control.

Discussion and Conclusions

The results of this study demonstrate that the Spartan DX is capable of generating accurate real-time PCR results for the detection of S. aureus in less than 30 min. By combining the capabilities of the Spartan DX with a simple DNA extraction method, it becomes possible to rapidly and accurately identify S. aureus infections using clinical isolates.

References

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