

The Benefit of Broth Enrichment Prior to PCR for the Detection of *S. aureus* from Nasal Swabs Obtained for Surveillance

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Amended Abstract

Introduction: Screening for *S. aureus* has become commonplace. The detection of the *sa442* genetic element by PCR has been shown to be a reliable method to detect the presence of *S. aureus*. We sought to compare the performance of the *sa442* gene PCR for the detection of *S. aureus* on direct lysates of nasal swabs with the same assay performed on lysates obtained following broth culture enrichment.

Materials & Methods: 250 nasal swabs were collected from patients prior to cardiothoracic surgery, and from patients admitted to the medical intensive care unit and once per week thereafter. Nucleic acids were obtained from the swab contents using a lysis procedure that included achromopeptidase. Aliquots of the lysates were tested for the presence of *S. aureus* DNA using the *sa442* gene PCR. The second swab was inoculated into enrichment broth, which was incubated for 18 hours. An aliquot of the enrichment broth was then tested for the presence of *S. aureus* using the same *sa442* gene PCR. The broth was subsequently subcultured for *S. aureus* to resolve discrepancies between the direct and broth-enriched PCR assays.

Results: 56 specimens contained *S. aureus*, whereas 194 specimens did not. The sensitivity, specificity, positive and negative predictive values for the two assays were as follows:

Assay	Sensitivity	Specificity	PPV	NPV
Sa442 Direct	91%	95%	84%	97%
Sa442 Broth Enrichment	100%	98%	95%	100%

Discussion/Conclusion: The sensitivity and specificity of the *sa442* gene PCR was improved, when a pre-PCR broth enrichment step was added. Broth enrichment yielded more true positives (9%; 5/56), fewer false negatives and fewer false positives compared with direct specimen testing. The drawback of broth enrichment PCR is the time-to-result delay. Future studies should investigate the minimum time of broth enrichment necessary to achieve a substantially increased detection of *S. aureus*.

Introduction

- Detection of the *sa442* genetic element by PCR has proven to be a reliable method for diagnosing the presence of *S. aureus* in clinical specimens
- During the period 2005-2006 compared to 2004-2005 at our institution, there was an observed 7% increase in positive *S. aureus* results from nasal swabs used to screen patients prior to cardiothoracic surgery. We had implemented a broth enrichment step to our PCR detecting the *sa442* gene in 2005 which may have explained this significant increase.

- We thus sought to compare the performance of the *sa442* gene PCR on direct lysates of nasal swabs versus its performance on lysates obtained following broth culture enrichment.

Materials and Methods

A total of 250 nasal swabs (COPAN Amies Double Swabs) were obtained from patients who were either being screened preoperatively for cardiothoracic surgery or had been admitted to the medical intensive care unit where swabs were obtained from patients on admission and weekly thereafter.

Swab 1

The first swab was broken off and inoculated into a microcentrifuge tube containing 300 µL of achromopeptidase (1 U/µL) in 1x TE buffer¹.

Tube was vortexed for 5 to 10 s and incubated at 37°C for 15 min followed by a second incubation at 99°C for 5 min. Samples were then stored at 4°C to cool.

After a quick spin, an aliquot (5 µL) of the lysate was tested for the presence of *S. aureus* DNA using the *sa442* gene PCR.

The *Sa442* gene was amplified on a LightCycler using previously described primers.^{2,3}

Sa-442-F (5'-GTCTGATACACGATATCTTCACG-3')

Sa-442-R (5'-CTCTCGTATGACCAGCTTCGGTAC-3')

Product detection was performed using previously described hybridization probes labeled with fluorescein (*fam*) and (*Red 705*) fluorophore molecules.

FRET Probes:

Sa442-HP-1 (5'-TACTGAAATCTCATTACGTTGCATCGGAA-[*fam*]³)

Sa442-HP-2 (5'[*Red 705*]-ATTGTGTTCTGTATGTAAAAGCCGCTCTTG-p3')

Swab 2

The second swab was inoculated into a tube of BBL™ Brain Heart Infusion (BHI) broth and incubated for 18 hours at 37°C.

BHI broth was then vortexed and a 50 µL aliquot placed into a microcentrifuge tube where 300 µL of achromopeptidase lysis buffer was added. Steps 2-4 for swab 1 were then repeated on swab 2 ending in amplification of the *sa442* gene using the same assay and primers.



COPAN Amies Double Swab



PCR was performed on the LightCycler

Broth subculture

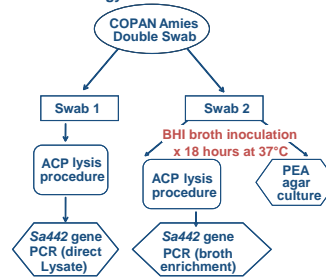
A 10 µL aliquot from the inoculated BHI broth was subcultured on PEA agar to resolve any discrepant results between the direct and broth-enriched PCR assays. Growth of *S. aureus* on the plate would equate to a positive result.

Data analysis

True/ composite positive = a specimen result that is uniformly positive by PCR on both direct lysate and enriched broth; in the event of a different result between the PCR on the direct lysate and the enriched broth, the broth subculture result was used to resolve discrepancies

True/ composite negative = a specimen that is uniformly negative by PCR on both direct lysate and enriched broth; in the event of a different result between the PCR on the direct lysate and the enriched broth, the broth subculture result was used to resolve discrepancies

Figure 1. Flowchart of methodology



LightCycler PCR Mixture for each sample:

- PCR grade H2O
- 3.0 mM MgCl₂
- 0.25 µM each forward and reverse *Sa442* primer
- 0.2 µM each *Sa442* FRET probe
- 2 µL of 10x LightCycler FastStart DNA Master Hybridization Probes
- 5 µL of template DNA extract
- Total volume of 20µL for each capillary tube

LightCycler Reaction Protocol

FastStart DNA Taq polymerase activation phase: (95°C for 10 minutes)
45-cycle amplification phase: (95°C for 10 seconds, 50°C for 10 seconds and 72°C for 20 seconds)

Melt phase: (45°C to 75°C with a temperature transition rate of 0.1°C/second)
Rapid cooling phase.

Results

- 56 specimens contained *S. aureus* whereas 194 specimens did not

Table 1A. Results of *sa442* PCR on direct lysates compared to the composite positive and negative results

	Composite positive	Composite negative
Sa442 Direct Positive	51	10
Sa442 Direct Negative	5	184

Table 1B. Results of *sa442* PCR following broth enrichment compared to the composite positive and negative results

	Composite positive	Composite negative
Broth enrichment positive	56	3
Broth enrichment negative	0	191

Table 2. Sensitivity and specificity, PPV and NPV of *sa442* gene Direct PCR vs *sa442* gene Broth Enrichment PCR

Assay	Sensitivity	Specificity	PPV	NPV
Sa442 Direct	91%	95%	84%	97%
Sa442 Broth Enrichment	100%	98%	95%	100%

Discussion / Conclusions

- Sensitivity and specificity of the *sa442* gene PCR was improved with addition of a pre-PCR broth enrichment step.
- Broth enrichment PCR yielded more true positives (5%; 3/56), fewer false negatives and fewer false positives compared with direct specimen testing
- The drawback of both enrichment PCR is the time to result delay. Future studies should investigate the minimum time of broth enrichment necessary to achieve a substantially increased detection of *S. aureus*

References

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