Highly Specific and Sensitive Detection and Quantification of *Staphylococcus aureus* Using Real-Time PCR

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Abstract

Staphylococcus aureus is a Gram-positive pathogen that can contaminate a variety of foods. *S. aureus* is found in meat, poultry, eggs, milk and dairy products, salads such as egg and tuna, and baked goods with cream fillings. Specific and sensitive detection of *S. aureus* has been achieved using an assay based on careful target selection and bioinformatics algorithms.

Purpose: The aim of this study was to develop a specific, reliable, and simple-to-use Real-Time PCR assay for *S. aureus* and demonstrate its utility for rapid and sensitive screening of enrichments of meat and dairy products.

Methods: A duplex Real-Time PCR assay was developed to detect *S. aureus* and a positive control sequence to monitor PCR inhibition. Assay performance was tested on an inclusivity panel of *S. aureus* isolates and an exclusivity panel of related *Staphylococcal* species. Sensitivity of the assay was evaluated using a quantified *S. aureus* DNA sample.

Results: The *S. aureus* assay, tested in lyophilized and tabletted form, detected 27 out of 27 (100%) *S. aureus* isolates in the inclusivity panel and none of the 25 (0%) species in the exclusivity panel. Limit of detection was estimated at 10 genomic copies of *S. aureus*. In naturally contaminated ground beef, *S. aureus* levels of 10 cfu per gram were detected within 24 hrs.

Significance: This study demonstrated that our assay specifically detects *S. aureus* and enables accurate identification of *S. aureus* from other *Staphylococcus* species. This specificity combined with the high sensitivity of the assay and ease-of-use would be amenable to *S. aureus* screening of diverse foods.

Assay design and features

The primers and probes in the *S. aureus* Real-Time PCR (TaqMan®) assay were designed using Applied Biosystems' rigorous bioinformatics pipeline that tabulates the effects of potential mismatches. The pipeline minimizes mismatches to inclusion targets while maximizing mismatches to non-targets. The algorithms were developed for production of Applied Biosystems' large collection of TaqMan® assays and have been extensively tested in-house to validate the assay design process.

The target selected by the assay design pipeline for *S. aureus* detection was determined to be unique in *S. aureus*. The assay was validated against an extensive inclusion and exclusion panel. The *S. aureus* assay is multiplexed with an internal positive control (IPC) which monitors PCR inhibition in each reaction.

The assay was tested in lyophilized and tabletted format. Dried-down formats consisted of PCR reagent components, primers, and probes. *Staphylococcus* species were enriched overnight, lysed, and lysates added to the tablets in a 30 µL reaction. The reactions were run on DuPont Qualicon's BAX Q7 system which produced plus/minus calls and generated Ct (cycle threshold) values.

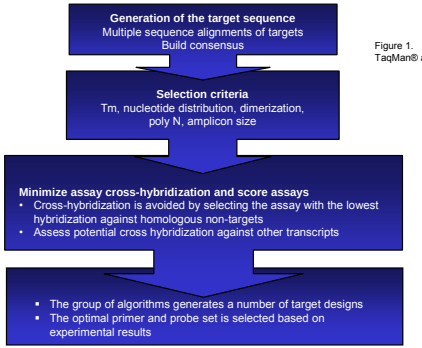


Figure 1.
TaqMan® assay design pipeline

Specificity

	Isolates	Pos	Neg
Inclusion			
Staphylococcus aureus	27	27	0
Exclusion			
Staphylococcus arlettae	1	0	1
Staphylococcus auricularl	1	0	1
Staphylococcus capitis	1	0	1
Staphylococcus caprae	2	0	2
Staphylococcus camosus	4	0	4
Staphylococcus caseolytic	1	0	1
Staphylococcus chromogenes	1	0	1
Staphylococcus cohnii	1	0	1
Staphylococcus delphini	1	0	1
Staphylococcus epidermidis	1	0	1
Staphylococcus felis	1	0	1
Staphylococcus gallinarum	1	0	1
Staphylococcus haemolyticus	1	0	1
Staphylococcus hominis	1	0	1
Staphylococcus intermedius	1	0	1
Staphylococcus saprophyti	1	0	1
Staphylococcus schleiferi	1	0	1
Staphylococcus sciuri	1	0	1
Staphylococcus simulans	1	0	1
Staphylococcus warneri	1	0	1
Staphylococcus xylosus	1	0	1

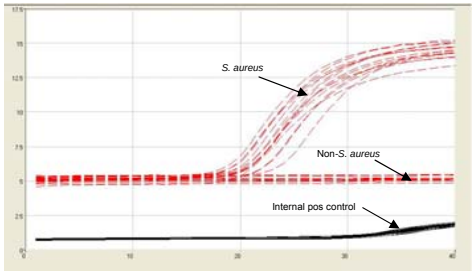
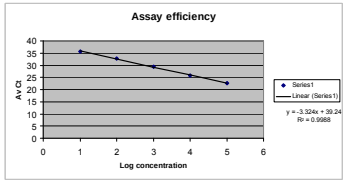
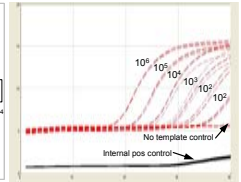


Figure 2. Amplification plots from *S. aureus* and non-*S. aureus* samples from the inclusion and exclusion panel.

Table 1. The *S. aureus* assay was specific for all isolates of *S. aureus* in the inclusion panel tested. This included the detection of an isolate of a methicillin-resistant strain of *S. aureus*. None of the closely related *Staphylococcus* species in the exclusion panel were detected.

Efficiency





Figures 3 and 4. Assay efficiency was determined by testing the assay over a dilution series of purified *S. aureus* genomic DNA. Each ten-fold DNA dilution was tested in quadruplicate. Efficiency was measured using the Ct slope method (plot of average Ct versus log DNA concentration). The expected slope for a ten-fold dilution series of DNA is -3.32 when efficiency is equal to 100% and would indicate a doubling of PCR product is occurring at every PCR cycle. The *S. aureus* assay demonstrated efficiency close to 100%.

Sensitivity

S. aureus gDNA (pg)	Genomic copy number	Av Ct
3000	1,000,000	22.58
300	100,000	25.83
30	10,000	29.40
3	1,000	32.83
0.3	100	35.70
0.03	10	37.43

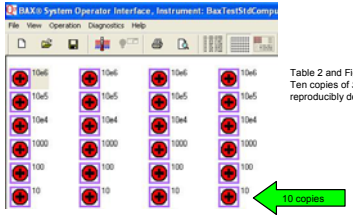


Table 2 and Figure 5.
Ten copies of *S. aureus* genomic DNA were reproducibly detected.

Comparison of Real-Time PCR to ISO reference method

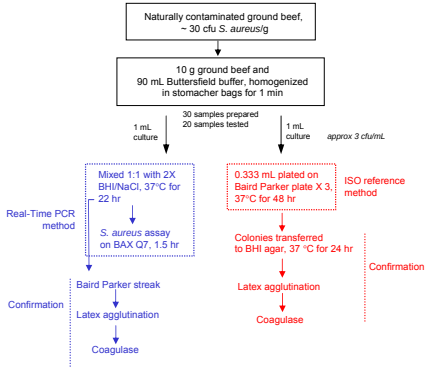


Figure 6 and Table 3. Sensitivities of Real-Time PCR and the ISO reference method for detection of *S. aureus* were compared. Equivalent test samples were taken from twenty different 10 g portions of *S. aureus*-contaminated ground beef mixed with pre-warmed Buttersfield buffer. The 1 mL test samples were separately (1) enriched in BHI/NaCl followed by lysis and Real-Time PCR and (2) plated on Baird Parker plates. Both Real-Time PCR and plating results were confirmed by latex agglutination and coagulase tests. The 1 mL test sample used for each method was estimated to contain on average 3 cfu *S. aureus*. Due to sampling distribution, it is expected that some samples would contain 0 cfu and would result in a negative result. The data in Table 3 show that the sensitivities of Real-Time PCR (18/20) and the ISO reference method (19/20) were comparable. Estimated concentrations of the master sample were based on the test samples and are indicated in the final column of Table 3.

	Real-time PCR BHI/NaCL				ISO			
Sample	BAX	BP streaks	latex aggl	coagulase	BP	latex aggl	coagulase	cfu/g
1	POS	POS	POS	POS	4+	4+	4+	40
2	POS	POS	POS	POS	5+	5+	5+	50
3	POS	POS	POS	POS	6+	6+	6+	60
4	POS	POS	POS	POS	4+	4+	4+	40
5	POS	POS	POS	POS	3+	3+	3+	30
6	POS	POS	POS	POS	4+	4+	4+	40
7	neg	neg			5+	5+	5+	50
8	POS	POS	POS	POS	3+	1+	1+	10
9	POS	POS	POS	POS	4+	3+	3+	30
10	POS	POS	POS	POS	4+	2+	2+	20
11	POS	POS	POS	POS	4+	2+	1+	10
12	POS	POS	POS	POS	4+	3+	3+	30
13	POS	POS	POS	POS	4+	4+	3+	30
14	POS	POS	POS	POS	3+	3+	1+	10
15	POS	POS	POS	POS	0+	0+	0+	
16	POS	POS	POS	POS	2+	0+	0+	
17	POS	POS	POS	POS	3+	3+	3+	30
18	POS	POS	POS	POS	4+	4+	4+	40
19	POS	POS	POS	POS	5+	1+	1+	10
20	neg	neg			3+	3+	3+	30
	18/20	18/20	18/20	18/20	19/20	18/20	18/20	

Conclusions

- A highly specific TaqMan® assay for the detection of *S. aureus* was developed using Applied Biosystems' assay design pipeline.
- The sensitivity of the assay permits reliable detection of 10 genomic copies of *S. aureus*.
- The sensitivity of detection of *S. aureus* in contaminated ground beef is comparable to the sensitivity of the ISO reference method.
- The time to result–including enrichment, sample prep, and Real-Time PCR–is within 24 hrs.
- The available tabletted form enables ease-of-use for rapid screening.

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