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 other foods. 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 enrichment and meat samples.

Methods

A specific 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 45 S. aureus isolates and an exclusivity panel of related Staphylococcus 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 20 (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/g per gram were detected within 24 hrs.

Significance

This study demonstrates that our assay specifically detects S. aureus and accurately identifies S. aureus from other Staphylococcus species. The 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 for the assay design was the 16S rRNA gene for S. aureus. 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 and, and lysates added to the tablets in a 30 μL reaction. The reactions were run on DuPont QuanTrak’s BAX Q7 system which produced real-time PCRs and generated Cq (cycle threshold) values.

Efficiency

Assay efficiency was determined by testing the assay over a tenfold DNA dilution series of purified S. aureus genomic DNA. Each ten-fold DNA dilution was tested in quadruplicate. Efficiency was measured as the slope of the linear regression plot for the cycle number versus log DNA concentration. The expected slope for a ten-fold dilution series of DNA is -3.324. The S. aureus assay demonstrated efficiency close to 100%.

Sensitivity

The S. aureus assay was evaluated for detection 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 exclusivity panel were detected.

Figure 1. TaqMan assay design pipeline

Figure 2. Amplification plots from S. aureus and non-S. aureus samples from the inclusion and exclusivity panels.

Figure 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 as the slope of the linear regression plot for the cycle number versus log DNA concentration. The expected slope for a ten fold dilution series of DNA is -3.324 when efficiency is equal to 100% and would indicate a doubling of PCR targets.

Figure 5. Time to result for different S. aureus constructs.

Table 3.

Table 3. Sensitivity of Real-Time PCR to ISO reference method

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<thead>
<tr>
<th>Sample</th>
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<th>Latex aggl</th>
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Conclusions

A highly specific TaqMan® assay for the detection of S. aureus was developed 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.

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