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Research Article

MICROBIOLOGICAL METHODS

Validation of the Thermo Scientific™ SureTect™ *Staphylococcus aureus* PCR Assay for the Detection of *Staphylococcus aureus* in Dairy Matrixes: AOAC *Performance Tested Method*℠ 052101

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Abstract

Background: The Thermo Scientific™ SureTect™ *Staphylococcus aureus* PCR Assay is a real-time PCR assay for the detection of *Staphylococcus aureus* in dairy samples.
Objective: The Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay was evaluated for AOAC *Performance Tested Method*℠ certification.
Methods: Inclusivity/exclusivity, matrix studies, product consistency and stability, and robustness testing were conducted to assess the method's performance. For the matrix study, the method was validated on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and the Applied Biosystems 7500 Fast Real-Time PCR instrument against the ISO 6888-3:2003 Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)—Part 3: Detection and MPN technique for low numbers, and the U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Ch. 12, *Staphylococcus aureus*, 2016, reference methods.
Results: Matrix studies showed no statistically significant differences between the candidate and reference methods or between presumptive and confirmed results. The inclusivity/exclusivity study correctly identified/excluded all strains analyzed. Robustness testing showed no statistically significant difference in assay performance after set method parameter deviations, and product consistency and stability studies demonstrated no statistically significant differences in performance between kit lots at different expiration points.

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Conclusion: The data presented show that the assay is a rapid and reliable workflow for the detection of *S. aureus* from dairy matrixes.
Highlights: The PCR assay allows for fast, reliable detection of *S. aureus* in dairy matrixes with results obtained in as little as 80 min post enrichment.

General Information

Staphylococcus aureus is recognized globally as a causative agent in a wide variety of diseases in humans and from zoonotic sources. It is considered one of the most significant etiological agents of mastitis infection within dairy cattle, which is of marked importance regarding food protection and public health (1). There are approximately 240 000 cases of staphylococcal food poisoning reported in the United States each year, although this is not truly representative of the incidence rate as sporadic disease caused by *S. aureus* is not reported (2). *S. aureus* is one of the most significant foodborne pathogens due to numerous varieties of heat-stable enterotoxins that the bacterium can produce leading to severe disease, as well as strains that express multi-drug resistant (MDR) genes leading to difficulty in treating both clinical cases and the zoonotic source of the infection (3).

Rapid detection of *S. aureus* from dairy food sources is of marked importance in protecting public health and ensuring fast throughput of production factories to ensure that food batches are released quickly and free of pathogens. Culture media-based reference methods can take up to 48 h to confirm suspected *S. aureus* colonies. The SureTect™ *Staphylococcus aureus* PCR workflow is designed to detect the presence of *S. aureus* from dairy samples in as few as 23 h.

Principle of the Method

The SureTect *Staphylococcus aureus* PCR Assay is used in conjunction with either the Applied Biosystems™ 7500 Fast Real-Time PCR instrument with Applied Biosystems RapidFinder Express Software (version 2.0 or higher) or the Applied Biosystems QuantStudio™ 5 Real-Time PCR instrument with Applied Biosystems RapidFinder Analysis Software (version 1.1 or higher) for the detection of *S. aureus* from dairy samples (4).

The assay is supplied as a kit containing all necessary reagents to conduct the sample lysis, including prefilled lysis tubes and lyophilized PCR pellets, containing all necessary PCR reagents (target-specific primers, dye-labelled probes, and PCR master mix components) to easily conduct the PCR analysis of the sample. PCR probes are short oligonucleotides with a quencher molecule at one end that, when not bound to target DNA, greatly reduces fluorescence from the dye label at the opposite end of the probe molecule. The oligonucleotides target unique DNA sequences unique to *S. aureus*. If *S. aureus* is present, the target DNA sequences will be amplified and the increasing fluorescent signal generated will be detected by the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument and interpreted by the respective software.

In addition to detection of any target DNA, the PCR pellets contain probes, primers, and DNA templates for an internal positive control (IPC). During PCR cycling, the IPC template is amplified regardless of the presence of the target DNA. The probe used for the IPC is labelled with a different colored fluorescent dye to the probes used within the assay to detect target DNA, and so can be detected by either the 7500 Fast Real-Time PCR instrument or the QuantStudio 5 Real-Time PCR instrument through a separate dye channel. If there is no presence of target DNA, the presence of the IPC amplification curve indicates that the PCR process has occurred successfully.

The PCR probes used in the SureTect *Staphylococcus aureus* PCR Assay are based on TaqMan™ PCR technology. Results are achieved approximately 80 min after loading the prepared sample into either PCR instrument and are displayed via the appropriate instrumentational software on the attached computer screen as simple positive or negative symbols with an attached PCR amplification plot that is easily accessible for review. All results interpreted by the software can be reported, stored, printed, and downloaded as required by the user.

Scope of Method

(a) *Analyte.*—*Staphylococcus aureus*.
(b) *Matrixes.*—Whey protein concentrate, whole milk powder, probiotic powdered infant formula, Edam cheese, and mozzarella cheese (up to 100 g portions).
(c) Summary of validated performance claims.—No statistically significant difference compared to the ISO 6888-3:2003 Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)—Part 3: Detection and MPN technique for low numbers after probability of detection (POD) analysis (5) and the U.S. Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM), Chapter 12 (2016), *Staphylococcus aureus* (6) reference methods.

Definitions

Example definitions for qualitative methods:

(a) *Probability of detection (POD).*—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated; POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD), and POD_{CC} (candidate method confirmation result POD).
(b) *Difference of probabilities of detection (dPOD).*—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

Materials and Methods

Test Kit Information

(a) *Kit name.*—Thermo Scientific™ SureTect™ *Staphylococcus aureus* PCR Assay.
(b) *Cat. no.*—A44255.

(c) *Ordering information.*
  (1) *USA.*—Remel Inc., part of Thermo Fisher Scientific, Santa Fe Dr, Lenexa, KS, 66215. Tel: (800) 255-6730.
  (2) *Europe.*—Oxoid Ltd, part of Thermo Fisher Scientific, Wade Rd, Basingstoke, Hampshire, RG24 8PW, UK. Tel: +44 1256 841144.
  (3) *Asia/Pacific/China.*—Thermo Fisher Scientific, Thermo Fisher Biochemicals (Beijing) Ltd, 3rd Floor, 28 Yuhua Rd, Area B, Tianzhu Airport Industrial Zone, Beijing 101312, China.
  (4) *Australia.*—Thermo Fisher Scientific Australia Pty Ltd, 20 Dalgleish St, Thebarton, Adelaide, South Australia, 5031.

Test Kit Components

(a) *Lysis Reagent 1 Tubes (clear, pale blue liquid containing fine white particles).*—Twelve strips of eight tubes.
(b) *Lysis Reagent 2 Tubes (clear, colorless liquid).*—Twelve strips of eight tubes.
(c) *Lysis Tube Caps, domed.*—Twelve strips of eight caps.
(d) *Proteinase K (clear colorless liquid).*—One tube.
(e) *SureTect Staphylococcus aureus PCR Tubes (PCR tubes).*—Twelve strips of eight tubes containing one pellet each.
(f) *PCR Caps.*—Twelve strips of eight caps.

Additional Supplies and Reagents

(a) *Oxoid™ Giolitti-Cantoni Broth.*—CM0523B or equivalent.
(b) *Potassium Tellurite supplement.*—SR0030 or equivalent.
(c) *Tween 80.*—Available from thermofisher.com.
(d) *Baird-Parker (BP) Agar Base (Dehydrated), 500 g.*—CM0275B or equivalent.
(e) *Egg Yolk Tellurite Emulsion 100%, 10 mL, 10/Pk.*—R450332.
(f) *Brilliance™ Staph 24 Agar, ready poured plates, 10 × 90 mm plates.*—PO1186A.
(g) *Thermo Scientific™ Staphaurex™ Latex Agglutination Test.*—R30859901 (120), R30859902 (400).

Equipment and Apparatus

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or thermofisher.com, MLS: Fisher Scientific (fisherscientific.com), or other major laboratory suppliers.

(a) *Homogenizer laboratory blender or dilutor, one of the following or equivalent.*—DB5000A, DB4100A, or DB4150A.
(b) *Homogenizer bags appropriate for the sample type and size.*—For DB4100A or DB4150A: DB4011A, DB4012A, DB4013A, DB4014A.
(c) *Incubator fitted with racks for homogenizer bags, set to 37±1°C.*
(d) *Disposable gloves.*
(e) *Variable volume single-channel pipet (10–100 μL).*
(f) *96-well rack.*—For holding samples.
(g) *Filtered pipet tips (10–100 μL).*
(h) *Sample tubes, 1.5 mL.*
(i) *Single-channel pipet or electronic adjustable spacing, multichannel pipet (10–100 μL).*
(j) *Single-channel stepper pipet (10–100 μL).*
(k) *Filtered pipet tips (10–100 μL).*
(l) *Thermo Scientific™ CapEase™ tool for capping and decapping lysis tubes.*—PT0621.
(m) *Applied Biosystems™ SimpliAmp™ Thermal Cycler.*—A24811.
(n) *MicroAmp™ 96-Well Tray/Retainer Set for Veriti™ Systems.*—4381850.
(o) *MicroAmp™ Splash-Free 96-Well Base.*—4312063.
(p) *QuantStudio™ 5 Food Safety Real-Time PCR Instrument, 0.1-mL block, with RapidFinder™ Analysis Software v1.1 or later. For use with SureTect™ Staphylococcus aureus PCR Assay and Pathogen Assay File: S.aureus SureTect QS5 version 1.0 or later.*—A36320 (desktop), A36328 (laptop).
(q) *7500 Fast Food Safety Real-Time PCR Instrument with RapidFinder™ Express Software v2.0 or later. For use with SureTect™ Staphylococcus aureus PCR Assay and Pathogen Assay File: S.aureus SureTect 1.0 or later.*—A30304 (desktop), A30299 (laptop).
(r) *Vortex mixer.*
(s) *Eight-channel pipet (10–100 μL).*
(t) *Filtered pipet tips (10–100 μL).*
(u) *MicroAmp™ 96-Well Tray for Veriflex™ Block.*—4379983.
(v) *MicroAmp™ Splash-Free 96-Well Base.*—4312063.
(w) *Precision Plate holder for SureTect™ assays or 7500 Fast Precision Plate holder, for 0.1 mL tube strips.*—PT0690 or A29252.
(x) *PCR Carry plate for SureTect™ assays.*—PT0695.
(y) *If using Precision Plate holder for SureTect™ assays.*—Cat. No. PT0690.
  (1) *VersiPlate PCR Strip Tube Plate, 96-well, low profile.*—AB1800.
  (2) *Ultra-Clear qPCR Caps, strips of 8.*—AB0866.
(z) *If using 7500 Fast Precision Plate Holder, for 0.1 mL tube strips.*—Cat. No. A29252.
  (1) *MicroAmp™ Fast 8-Tube Strip, 0.1 mL.*—4358293.
  (2) *MicroAmp™ Optical 8-Cap Strips.*—4323032.

Standard Reference Materials

(a) *Research and Development Culture Collection (RDCC).*—Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK,
(b) *Trials Culture Collection (TCC).*—Thermo Fisher Scientific, Microbiology Division, Basingstoke, UK,
(c) *National Collection of Type Cultures (NCTC).*—Salisbury, United Kingdom.
(d) *American Type Culture Collection (ATCC).*—Manassas, VA.

Safety Precautions

General safety: Using the PCR kit in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practice for laboratories and the safety information provided in this document. Before handling chemicals, read and understand all applicable Safety Data Sheets (SDS) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.).

Refer to the 7500 Fast Real-Time PCR System and QuantStudio 5 Real-Time qPCR System Instrument Manuals for guidelines on cleaning equipment and handling possible amplicon contamination. For disposal of uninoculated culture media or any reagents and materials included in the SureTect Staphylococcus aureus PCR Assay and associated tests, refer to the manufacturers material SDS and apply appropriate local guidelines.

To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions. Read and understand the

SDS provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain the SDS, see the "Documentation and Support" section in this document. Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood). Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS. Handle chemical wastes in a fume hood. Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste, a secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.) After emptying a waste container, seal it with the cap provided. Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory. Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Depending on the samples used on this instrument, the surface may be considered a biohazard.

Use appropriate decontamination methods when working with biohazards. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations.

S. aureus may be present in many human specimens and in food. The primary hazards to laboratory personnel are direct and indirect contact of broken skin or mucous membranes with cultures and contaminated laboratory surfaces, parenteral inoculation, and ingestion of contaminated materials. BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infected clinical materials or cultures. The following references provide general guidelines when handling biological samples in a laboratory environment:

(a) U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020; found at: Biosafety in Microbiological and Biomedical Laboratories—6th Edition (cdc.gov).

(b) World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at: https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf.

General Preparation

(a) Prepare all media and use all confirmation kits according to the manufacturer's instructions.

(b) Turn on the SimpliAmp Thermal Cycler and ensure that the required temperature cycle is set according to the manufacturer's instructions.

(c) Allow Lysis Reagent and PCR tubes to reach ambient temperature (23 ± 5°C) before use by placing on the laboratory bench about 10 min before they are required.

Sample Preparation

The enrichment procedure is consistent for all matrixes claimed (powdered infant formula, whole milk powder, whey protein concentrate, Edam cheese, and mozzarella cheese): For 100 g test portions, 1-in-10 ratio of sample to media. Add 900 mL of Giolitti-Cantoni Broth with Potassium Tellurite supplement (0.01% per 225 mL) and Tween 80 (1 g per 1000 mL). Homogenize the sample for 30 s to 1 min using a homogenizer and incubate at 37.0 ± 1°C for 22–26 h.

Analysis

Lysis

(a) Equilibrate the Lysis Reagent 1 Tubes to room temperature (23.0 ± 5°C).
   (1) Place the required number of Lysis Reagent 1 Tubes in a MicroAmp Splash-Free 96-Well Base and MicroAmp 96-Well Tray.
   (2) Check that there is no liquid around the plastic seal and the reagents are collected at the bottom of each tube.
   (3) Allow the tubes to remain at room temperature for approximately 10 min before opening.

(b) Remove the plastic seal from each Lysis Reagent 1 Tube, then add 10 µL of Proteinase K and Lysis Reagent 2 to the tube. Avoid contamination of the Proteinase K stock tube by using a new filtered pipet tip each time Proteinase K is withdrawn from the stock tube. Use a 10–100 µL repeat pipettor to reduce the number of tips required.

(c) Transfer 10 µL of the enriched sample to a Lysis Tube. For the negative extraction controls, transfer 10 µL of sterile enrichment media to a Lysis Tube. Ensure that the pipet tip reaches the bottom of the Lysis Tube to facilitate complete mixing of the sample with Lysis Reagent 1.

(d) Seal the tubes with domed Lysis Tube Caps. To conduct the lysis procedure, incubate the samples in the Biosystems SimpliAmp Thermal Cycler set to the parameters outlined in Table 1. *Note:* To prevent crushing of the tubes in the Biosystems SimpliAmp Thermal Cycler, use the bottom piece from the MicroAmp 96-Well Tray/Retainer Set, or include at least four complete SureTect Lysis tube strips.

PCR set-up—QuantStudio 5 instrument

(a) Use the RapidFinder Analysis Software to determine the Run Layout (plate layout) for samples based on the information entered and create a run file. Refer to the online help in the software for more details.

Table 1. Applied Biosystems SimpliAmp thermal cycler run parameters

Step	Temperature, °C	Time, min
Lid heater	105	Maximum time
1	37	10
2	95	5
3	10	2
4	4	Hold[a]

[a] For convenience, samples can be held at 4°C until proceeding to PCR or transfer to storage at 2–8°C.

(b) On the home page of the RapidFinder Analysis Software, select "Create Experiment" then "enter" or "edit" the well parameters. Select S.aureus QS5 version 1.0 (or later) for the assay.
(c) Following the plate layout previously set up in the software, place the required number of PCR tubes in the MicroAmp 96-Well Tray for VeriFlex Block. Place the block on the MicroAmp Splash-Free 96-Well Base. Press the PCR tubes to the tray to ensure they sit firmly, then tap the tubes on the bench to ensure that the pellets are located at the bottom of the tubes.
(d) Allow the PCR tubes to remain on the bench for approximately 5 min, to bring to room temperature, then open one strip of PCR tubes by removing the seal.
  (1) PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
  (2) If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty pipet tip. Do not use a tip containing lysate.
(e) Uncap the Lysis Tubes using the decapping tool.
(f) Transfer 20 μL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet. Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube as this can inhibit PCR from occurring—also ensure not to touch the pellet when adding the lysate.
(g) Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.
(h) Mix all the PCR tubes thoroughly by vortexing for 10–15 s to ensure that the pellet is fully rehydrated. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright, and flick sharply downwards.
(i) Start the run within 30 min of the addition of the sample lysates to the PCR tubes.
(j) Eject the instrument drawer. Use the MicroAmp 96-Well Tray for VeriFlex Block to transfer the tubes to the instrument in the same configuration as the plate layout determined in the software, then close the instrument drawer.
(k) In the "Run" tab of the experiment file in RapidFinder Analysis Software, select the instrument's serial number from the Instrument drop-down list.
(l) Click "Start Run," then follow the software prompts.

PCR set-up—7500 fast instrument

(a) Use the RapidFinder Express Software to determine the Run Layout (plate layout) for samples based on the information entered and create a run file. Refer to the online help in the software for more details.
(b) On the main page of RapidFinder Express Software, select "Create/Edit a Run File," then "enter" or "edit" the Run File information at the prompts. Plate layouts can be manually customized in the software. Select "S.aureus SureTect 1.0 or later" for the assay.
(c) Following the plate layout previously set up in the software, place the required number of PCR tubes in a suitable rack with a PCR carry plate, then tap the rack of tubes on the bench to ensure that the pellets are located at the bottom of the tubes. If required by the plate layout, place empty PCR tubes in the rack to balance the tray when the tubes are placed in the instrument.
(d) Allow the PCR tubes to remain on the bench for approximately 5 min, to bring to room temperature, then open one strip of PCR tubes by removing the seal.
  (1) PCR pellets are pale yellow. If the pellet is collapsed or not pale yellow, do not use.
  (2) If the pellet is not positioned at the bottom of a tube, gently move the pellet to the bottom of the tube with a sterile, empty pipet tip. Do not use a tip containing lysate.
(e) Uncap the Lysis Tubes using the decapping tool.
(f) Transfer 20 μL of the lysate or mock-purified sample (negative extraction control reaction) to the appropriate PCR tube to rehydrate the pellet. Tap the rack to ensure that the lysate is at the bottom of the tube and touching the pellet. Remove lysate from the top half of the liquid to ensure that no lysis particles are transferred from the Lysis Tube to the PCR tube as this can inhibit PCR from occurring. Do not touch the pellet when adding the lysate.
(g) Seal the PCR tubes with the flat optical PCR Caps provided with the kit. Ensure that the tubes are properly sealed by pressing down firmly over each opening. Do not use the capping tool to seal the PCR tubes.
(h) Mix all PCR tubes thoroughly by vortexing for 10–15 s to ensure that the pellet is fully rehydrated. Ensure that the liquid is at the bottom of the tube before placing in the PCR instrument. If needed, hold the tubes upright and flick sharply downwards.
(i) Start the PCR run within 30 min of addition of sample lysates to the PCR tubes.
(j) In the RapidFinder Express Software, select "Start Instrument Run" on the main page, select the appropriate run file, and follow the software prompts.
(k) Use the PCR carry plate to transfer the tubes to the instrument in the same configuration as the run layout. Use the Precision Plate Holder for SureTect assays. Load empty SureTect PCR tube strips as directed by the software.
(l) Close the tray to the instrument and follow the RapidFinder Express Software prompts to start the run.

Interpretation and test result report.—For the QuantStudio 5: Data analysis is automated by the software. For detailed instructions, and options for reporting, export, and storage of results, see the "Help" function in the software. In the home screen of the RapidFinder Analysis Software, click "Results", then click the sub-tab for the desired view of the data.

"Summary—plate format
Results—table format
Details—amplification plot

For the 7500 Fast: Data analysis is automated by the software. In the RapidFinder Express Software, select "View Results" on the main page, select the appropriate run file, and follow the prompts to view results. To display a list of results in table format, click "Table View". Select a sample, then click "View Details" to see replicate information about samples.

Confirmation.—Samples with positive PCR results must be confirmed by selective plating and the use of a latex test or by using conventional tests described in the methods standardized by CEN or ISO (EN ISO 6888-3:2003) or the FDA/BAM

Chapter 12 from colonies (including the purification step). The confirmation step must start from the primary enrichment broth.

To confirm presumptive positives using alternative confirmation method:

(1) Streak 10 μL of enriched sample onto BP agar or *Brilliance* Staph 24 Agar.
(2) Incubate at 37°C for 20–24 h for *Brilliance* Staph 24 Agar or 48 h for BP.
(3) Confirm presumptive positive samples using the Thermo Scientific Staphaurex Latex Agglutination Test of reference method techniques (EN ISO 6888-3:2003).

Validation Study

This validation study was conducted under the AOAC Research Institute (RI) *Performance Tested Method(s)*℠ program and the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* (7). Method developer studies were conducted in the laboratories of Thermo Fisher Scientific, Basingstoke, UK, and Thermo Fisher Scientific Oy, Vantaa, Finland, and included the inclusivity/exclusivity study and product consistency and stability study, respectively. The independent laboratory study was conducted by Q Laboratories, Inc., Cincinnati, Ohio and included a matrix study for all five claimed dairy matrixes and a robustness study. The studies were conducted according to the AOAC guidelines for validation of microbiological methods following an unpaired study design.

Method Developer Studies

Inclusivity and exclusivity study.—A total of 50 inclusivity isolates of *S. aureus* were analyzed with the SureTect *Staphylococcus aureus* PCR Assay on the 7500 Fast and QuantStudio 5 instruments. Isolates were obtained from internal research and development culture collections with the origin of strains representing a wide range of food, clinical, and environmental sources.

Inclusivity testing was conducted by removing isolates from −80°C storage and culturing each strain in Giolitti-Cantoni Broth with Potassium Tellurite supplement and Tween 80 for 37 ± 1°C for 22 h. Each isolate was diluted, if necessary, in sterile enrichment media to approximately 10–11 times the LOD_{50} of the SureTect *Staphylococcus aureus* PCR Assay for testing.

A total of 51 exclusivity isolates were analyzed on the 7500 Fast and QuantStudio 5 instruments, comprising of closely related non-target bacterial species and non-target *Staphylococcus* species. Isolates were obtained from internal R&D culture collections. Exclusivity testing was conducted by removing isolates from −80°C storage, streaking onto tryptic soya agar plates, and then incubating at 18–24 h at 37 ± 1°C. Each isolate was then inoculated into nutrient broth and cultured for 18–24 h and 37 ± 1°C.

Inclusivity and exclusivity isolates were blind-coded and randomized to ensure unbiased processing of samples. All isolates tested were successfully detected or excluded on both instruments. The results for inclusivity and exclusivity are shown in Tables 2 and 3, respectively.

Product consistency (lot-to-lot) and stability studies.—The product consistency and stability study examined three lots of the SureTect *Staphylococcus aureus* PCR Assay for lot-to-lot variable and product stability to confirm that the manufacturing and performance of the kits were consistent at different points during shelf life. The three kits tested consisted of one lot near the expiration date (END), one near the middle of the expiration period (MIDDLE), and one lot that had been recently manufactured (FRESH).

A strain of *S. aureus* ATCC 27664™ was cultured in Giolitti-Cantoni Broth with Potassium Tellurite supplement and Tween 80 at 37 ± 1°C for 22 h to act as a target organism for comparison between the three kits. This was then diluted in sterile enrichment buffer to a concentration at the limit of detection (LOD_{50}) of the SureTect *Staphylococcus aureus* PCR Assay to achieve fractional recovery.

A strain of *Staphylococcus epidermidis* ATCC 14990™ was cultured in Buffered Peptone Water (BPW) ISO at 37 ± 1°C for 24 h to act as a non-target organism for comparison between the three kits and was not diluted.

Each kit lot was tested with 10 replicates each of the target *S. aureus* strain and non-target *S. epidermidis* strain in a randomized blind coded fashion.

POD values and confidence intervals were calculated from the data to determine any statistical variation between lots. *S. epidermidis* samples were negative for all replicates for all three kits.

Fluorescence levels were comparable between kit lots and there was no significant difference between the kits at the 5% confidence level. The POD analysis of the stability study is outlined in Table 4.

Independent Laboratory Studies

Matrix study.—The matrix study was conducted according to the AOAC *Guidelines for Validation of Microbiological Methods* methods following the AOAC RI *Performance Tested Methods*℠ following an unpaired study design for 100 g whey protein concentrate, 100 g powdered infant formula (with probiotics), 100 g whole milk powder, 100 g mozzarella cheese, and 100 g Edam cheese. The Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay was compared to a modified version of the FDA/BAM Chapter 12 reference method and ISO 6888-3:2003 reference method. The FDA/BAM chapter 12 reference method was modified from the MPN enumeration format to a detection format, to allow for comparison to the candidate non-quantitative detection method. Since MPN methods are based on a series of qualitative methods, it was determined that using the qualitative method found in the FDA/BAM MPN for *S. aureus* method was appropriate. The modified method used the same enrichment as detailed in FDA/BAM Chapter 12, section B, and the enrichment ratio of sample to media was the same as outlined in FDA/BAM Chapter 1, Section G.1 (8). This modification allowed for direct comparison of the candidate qualitative method by altering to FDA reference methodology to detection but adhering to determined enrichment procedures and ratios.

In addition, an alternative confirmation was validated as previously outlined in the "*Confirmation*" section.

Whey protein concentrate, powdered infant formula (with probiotics), whole milk powder, mozzarella cheese, and Edam cheese were purchased from a local supplier and prescreened for natural contamination of *S. aureus* following the FDA/BAM Chapter 12 reference method. Total aerobic plate count was determined following the FDA/BAM Chapter 3 *Aerobic Plate Count* reference method (9). No natural contamination was detected in the screening of all five products. The matrixes were artificially contaminated as follows: *S. aureus* QL030911-4 was used to inoculate whey protein concentrate, *S. aureus* ATCC 11632™ was

Table 2. Inclusivity results of Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay

ID	Source	Origin	Strain name	Result
ATCC[a]	33591	Human—clinical	*Staphylococcus aureus*	+
RDCC[b]	0069	Unknown	*Staphylococcus aureus*	+
RDCC	0070	Unknown	*Staphylococcus aureus*	+
RDCC	0071	Unknown	*Staphylococcus aureus*	+
RDCC	122	Unknown	*Staphylococcus aureus*	+
ATCC	9144	Human—clinical	*Staphylococcus aureus*	+
RDCC	254	Unknown	*Staphylococcus aureus*	+
ATCC	12600	Pleural fluid	*Staphylococcus aureus*	+
ATCC	6538	Human lesion	*Staphylococcus aureus*	+
RDCC	502	Unknown	*Staphylococcus aureus*	+
ATCC	29737	Unknown	*Staphylococcus aureus*	+
NCTC[c]	7447	Unknown	*Staphylococcus aureus*	+
NCTC	13143	Unknown	*Staphylococcus aureus*	+
ATCC	43300	Human—clinical, Kansas	*Staphylococcus aureus*	+
NCTC	10442	Human—finger	*Staphylococcus aureus*	+
NCTC	12493	Human—clinical	*Staphylococcus aureus*	+
RDCC	900	Unknown	*Staphylococcus aureus*	+
RDCC	922	Unknown	*Staphylococcus aureus*	+
RDCC	923	Unknown	*Staphylococcus aureus*	+
RDCC	930	Unknown	*Staphylococcus aureus*	+
RDCC	931	Unknown	*Staphylococcus aureus*	+
RDCC	1105	Germany—human	*Staphylococcus aureus*	+
RDCC	1106	Germany—human	*Staphylococcus aureus*	+
RDCC	1645	Unknown	*Staphylococcus aureus*	+
ATCC	49476	Clinical	*Staphylococcus aureus*	+
RDCC	2953	Food Res Inst of Wisconsin	*Staphylococcus aureus*	+
RDCC	3053	Unknown	*Staphylococcus aureus*	+
RDCC	3901	Unknown	*Staphylococcus aureus*	+
RDCC	3903	Denka—Japan	*Staphylococcus aureus*	+
RDCC	3904	Oxoid, France	*Staphylococcus aureus*	+
RDCC	3917	Ex UCH	*Staphylococcus aureus*	+
RDCC	3918	Unknown	*Staphylococcus aureus*	+
RDCC	3919	Unknown	*Staphylococcus aureus*	+
RDCC	4011	Alfred Hosp, Melbourne—human	*Staphylococcus aureus*	+
RDCC	4012	Alfred Hosp, Melbourne—human	*Staphylococcus aureus*	+
RDCC	4658	Unknown	*Staphylococcus aureus*	+
RDCC	4659	Unknown	*Staphylococcus aureus*	+
RDCC	4761	Clinical	*Staphylococcus aureus*	+
RDCC	4762	Clinical	*Staphylococcus aureus*	+
RDCC	4763	Clinical	*Staphylococcus aureus*	+
RDCC	4764	Clinical	*Staphylococcus aureus*	+
RDCC	4765	Clinical	*Staphylococcus aureus*	+
RDCC	5058	US—wound swab (human)	*Staphylococcus aureus*	+
RDCC	5059	US—wound swab (human)	*Staphylococcus aureus*	+
RDCC	5060	US—wound swab (human)	*Staphylococcus aureus*	+
RDCC	5145	US—clinical swab	*Staphylococcus aureus*	+
RDCC	5268	Unknown	*Staphylococcus aureus*	+
NCTC	12497	Clinical	*Staphylococcus aureus*	+
NCTC	10345	Clinical	*Staphylococcus aureus*	+
RDCC	482	Unknown	*Staphylococcus aureus*	+

[a] ATCC = American Type Culture Collection, Manassas, VA, USA.
[b] RDCC = Thermo Fisher Scientific, Research and Development Culture Collection, Basingstoke, UK.
[c] NCTC = National Collection of Type Cultures, Salisbury, UK.

used to inoculate whole milk powder, *S. aureus* ATCC 25923™ was used to inoculate powdered infant formula, *S. aureus* ATCC 29737™ was used to inoculate mozzarella cheese, and *S. aureus* ATCC 33862™ was used to inoculate Edam cheese.

The matrix study consisted of evaluating a total of 30 unpaired 100 g portions for whey protein concentrate, whole milk powder, powdered infant formula, mozzarella cheese, and Edam cheese following an unpaired study design. Within each sample set, there were five uninoculated portions (0 CFU/test portion), 20 low-level inoculated portions (0.2–2 CFU/test portion), and five high-level inoculated portions (2–10 CFU/test portion). All test portions were analyzed by following the Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay workflow after 22 h of enrichment. All test portions were analyzed using the Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and 7500 Fast Real-Time PCR Instrument. All portions

Table 3. Exclusivity results of Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay

ID	Source	Origin	Strain name	Result
TCC[a]	627	Germany	Coagulase negative *Staphylococcus*	–
TCC	629	Germany	Coagulase negative *Staphylococcus*	–
TCC	632	Germany	Coagulase negative *Staphylococcus*	–
TCC	633	Germany	Coagulase negative *Staphylococcus*	–
TCC	634	Germany	Coagulase negative *Staphylococcus*	–
TCC	635	Germany	Coagulase negative *Staphylococcus*	–
TCC	636	Germany	Coagulase negative *Staphylococcus*	–
TCC	637	Germany	Coagulase negative *Staphylococcus*	–
TCC	638	Germany	Coagulase negative *Staphylococcus*	–
TCC	639	Germany	Coagulase negative *Staphylococcus*	–
TCC	640	Germany	Coagulase negative *Staphylococcus*	–
TCC	641	Germany	Coagulase negative *Staphylococcus*	–
TCC	642	Germany	Coagulase negative *Staphylococcus*	–
TCC	643	Germany	Coagulase negative *Staphylococcus*	–
TCC	644	Germany	Coagulase negative *Staphylococcus*	–
ATCC[b]	27853	Blood—human	*Pseudomonas aeruginosa*	–
ATCC	13525	Pre-filter water tanks, England	*Pseudomonas fluorescens*	–
RDCC[c]	2170	Unknown	*Pseudomonas putida*	–
ATCC	4973	Unknown	*Pseudomonas fragi*	–
RDCC	2977	Unknown	*Pseudomonas stuzeri*	–
RDCC	2980	Unknown	*Pseudomonas vesicularis*	–
RDCC	1415	Unknown	*Staphylococcus capitis*	–
RDCC	1789	Unknown	*Staphylococcus caprae*	–
ATCC	51365	Dry sausage	*Staphylococcus carnosus*	–
RDCC	1931	Grenoble, France	*Staphylococcus chromogenes*	–
RDCC	2812	King Georges Hospital, Redbridge	*Staphylococcus cohnii*	–
RDCC	669	Human (hospital)	*Staphylococcus epidermidis*	–
RDCC	670	Human (hospital)	*Staphylococcus epidermidis*	–
RDCC	1319	Unknown	*Staphylococcus haemolyticus*	–
RDCC	1320	Unknown	*Staphylococcus haemolyticus*	–
RDCC	986	Unknown	*Staphylococcus hominis*	–
RDCC	1162	Unknown	*Staphylococcus hominis*	–
RDCC	2695	Hospital Edouord Herriot, Lyon—human	*Staphylococcus hyicus*	–
RDCC	121	Human	*Staphylococcus intermedius*	–
RDCC	2897	Salmon	*Staphylococcus lentus*	–
RDCC	5621	Germany—culture media contaminant	*Staphylococcus lentus*	–
NCTC[d]	7990	Blood culture	*Staphylococcus lugdenensis*	–
ATCC	43809	Auxillary lymph node, France	*Staphylococcus lugdenensis*	–
RDCC	550	Veterinary isolate	*Staphylococcus pseudointermedius*	–
RDCC	551	Veterinary Isolate	*Staphylococcus pseudointermedius*	–
ATCC	15305	Urine—human	*Staphylococcus saprophiticus*	–
RDCC	1933	Limoges Hospital—human	*Staphylococcus schleiferi*	–
RDCC	2680	Hospital Edouord Herriot, Lyon—human	*Staphylococcus schleiferi*	–
RDCC	4379	Unknown	*Staphylococcus sciuri*	–
RDCC	4069	Basildon Hospital—human	*Staphylococcus simluans*	–
RDCC	1930	Unknown	*Staphylococcus simulans*	–
RDCC	1321	Unknown	*Staphylococcus warneri*	–
RDCC	1429	Unknown	*Staphylococcus warneri*	–
ATCC	29971	Human skin	*Staphylococcus xylosus*	–
RDCC	3748	Unknown	*Staphylococcus xylosus*	–
RDCC	2972	Unknown	*Stenotrophomonas* maltophila	–

[a] TCC = Internal Trials Culture Collection, Thermo Fisher Scientific, Basingstoke, UK.
[b] ATCC = American Type Culture Collection, Manassas, VA, USA.
[c] RDCC = Thermo Fisher Scientific, Research and Development Culture Collection, Basingstoke, UK.
[d] NCTC = National Collection of Type Cultures, Salisbury, UK.

regardless of presumptive results were culturally confirmed by the modified FDA/BAM Chapter 12 and ISO 6888-3:2003 reference methods. In addition, an alternative confirmation was performed as previously described in the section "*Confirmation*."

For the whey protein concentrate, whole milk powder, and powdered infant formula matrixes, inoculation was conducted with a lyophilized culture. The *S. aureus* cultures were propagated on tryptic soy agars with 5% sheep blood (SBA) from a stock culture stored at −70°C. SBA was incubated for 24 ± 4 h at 37 ± 1°C. A single colony was then transferred to brain heart infusion (BHI) broth and incubated for 24 ± 4 h at 37 ± 1°C. The culture was then diluted in a sterile cryoprotectant, reconstituted

Table 4. Product consistency (lot-to-lot) and stability of Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay

Kit lot No.	Lot age, months	N[a]	x[b]	POD_A[c]	95% CI	Kit lot No.	Lot age, months	N	x	POD_B[d]	95% CI	$dPOD_{AB}$[e]	95% CI[f]
Staphylococcus aureus ATCC 27664 (target)													
020002232	Fresh[g]	10	5	0.50	0.24, 0.76	'020002232	Middle	10	6	0.60	0.31, 0.83	0.10	−0.29, 0.45
020001962	Fresh	10	5	0.50	0.24, 0.76	'020001962	End[i]	10	7	0.70	0.40, 0.89	0.20	−0.20, 0.53
Lot 5	Middle[h]	10	6	0.60	0.31, 0.83	Lot 5	End	10	7	0.70	0.40, 0.89	0.10	−0.28, 0.45
Staphylococcus epidermidis ATCC 14990 (non-target)													
020002232	Fresh	10	0	0.00	0.00, 0.28	020002232	Middle	10	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
020001962	Fresh	10	0	0.00	0.00, 0.28	'020001962	End	10	0	0.00	0.00, 0.28	0.00	'−0.28, 0.28
Lot 5	Middle	10	0	0.00	0.00, 0.28	Lot 5	End	10	0	0.00	0.00, 0.28	0.00	'−0.28, 0.28

[a] N = Number of test portions.
[b] x = Number of positive test portions.
[c] POD_A = Positive outcomes divided by the total number of trials first member of pair.
[d] POD_B = Positive outcomes divided by the total number of trials second member of pair.
[e] $dPOD_{AB}$ = Difference in POD between the paired comparison.
[f] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[g] Fresh = 1 month old.
[h] Middle = 7 months old.
[i] End = 11 months old.

non-fat dry milk (NFDM), and freeze dried for 48–72 h. A bulk lot of the matrix was inoculated with a lyophilized culture that was diluted in powdered NFDM to a low level expected to yield fractional positive results (5–15 positive results), and a high level expected to yield all positive results. After inoculation, materials were held for 2 weeks at room temperature (20–25°C). To create the 100 g portions for the SureTect analysis, 10 g of inoculated product was added to 90 g of noninoculated product. To create the 50 g portions for the modified FDA/BAM Chapter 12 reference method, 10 g of inoculated product was added to 40 g of noninoculated product. For the ISO 6888-3:2003 reference method, individual 10 g test portions from the inoculated materials and the noninoculated material were aseptically weighed into sterile stomacher bags.

For the mozzarella cheese and Edam cheese matrixes a liquid heat stressed culture was used. The liquid heat stressed culture was prepared as follows. The *S. aureus* cultures were propagated on SBA from a stock culture stored at −70°C. SBA was incubated for 24 ± 4 h at 37 ± 1°C. The pure culture was transferred to BHI broth and incubated for 24 ± 4 h at 37 ± 1°C. Following incubation, the culture was heat stressed by heating the culture at 55 ± 0.1°C for 10–20 min. The heat stressed culture was plated to the non-selective agar (TSA) and a selective agar (BP) and incubated for 18–24 h at 35 ± 1°C. Following incubation, the percentage injury was determined using the following formula, and the inoculating culture must have a percentage injury of 50–80:

$$\left(1 - \frac{n_{select}}{n_{nonselect}}\right) x100$$

where n_{select} = number of colonies on selective agar; and
$n_{nonselect}$ = number of colonies on nonselective agar

The *S. aureus* levels were confirmed by performing ten-fold serial dilutions using phosphate buffered saline to obtain an approximate inoculation level. A bulk material of mozzarella cheese and Edam cheese was prepared by shredding each cheese to allow for mixing for homogenous inoculation. The bulk sample of each cheese was inoculated with an appropriate volume, as to not adversely affect the water activity of the sample and at a dilution which considered initial die off and achieved each of the desired contamination levels: a low level expected to yield fractional positive results (5–15 positive results), and a high level expected to yield all positive results. To create the 100 g test portions for the SureTect analysis, 10 g of inoculated material was added to 90 g of noninoculated material. To create the 50 g test portions for the modified FDA/BAM Chapter 12 reference method analysis, 10 g of inoculated material was added to 40 g of noninoculated material. For the ISO 6888-3:2003 reference method, individual 10 g test portions from the inoculated materials and the noninoculated material were aseptically weighed into sterile stomacher bags. Test portions were stored for 48–72 h at 2–8°C before being tested.

Most probable number analysis.—The concentration of *S. aureus* in the low-level inoculum for all 10 g test portions was determined by most probable number (MPN) on the day of analysis by evaluating 5 × 20 g, 20 × 10 g (ISO 6888-3:2003 reference method test portions), and 5 × 5 g inoculated test samples. The concentration of *S. aureus* in the high-level inoculum for all 10 g test portions was determined by MPN by evaluating 5 × 10 g (ISO 6888-3:2003 reference method test portions), 5 × 5 g, and 5 × 1 g inoculated test samples. To the 20 g portions 180 mL of the reference method enrichment broth was added, to the 5 g portions 45 mL of the reference method enrichment broth was added, and to the 1 g portions 9 mL enrichment broth was added.

The concentration of *S. aureus* in the low-level inoculum for all 50 g test portions was determined by MPN on the day of analysis by evaluating 5 × 100 g, 20 × 50 g (modified FDA/BAM Chapter 12 reference method test portions), and 5 × 25 g inoculated test samples. The concentration of *S. aureus* in the high-level inoculum for all 50 g test portions was determined by MPN by evaluating 5 × 50 g (modified FDA/BAM Chapter 12 reference method test portions), 5 × 25 g, and 5 × 10 g inoculated test samples. To the 100 g portions 900 mL of the reference method enrichment broth was added, to the 25 g portions 225 mL of the reference method enrichment broth was added, and to the 10 g portions 90 mL enrichment broth was added.

All 10 g portions were utilized from unpaired ISO 6888-3:2003 reference method test potions analyzed following the ISO 6888-3:2003 reference method. All 50 g portions were utilized from

unpaired FDA/BAM Chapter 12 reference method test portions analyzed following the FDA/BAM Chapter 12 reference method. The number of positives from the three test levels was used to calculate the MPN using the LCF MPN calculator (version 1.6) provided by AOAC RI (10).

Modified FDA/BAM Chapter 12 method.—The FDA/BAM Chapter 12 reference method was modified by not conducting an MPN using 1 mL portions of decimal dilutions, but instead, 50 g test portions were directly enriched with TSB containing 10% NaCl and 1% sodium pyruvate. This enabled qualitative comparison to the candidate method by modifying the reference method from enumeration to detection.

Enrichments were then tested for the presence or absence of *S. aureus*. For the FDA/BAM Chapter 12 reference method, 50 g test portions were enriched with 450 ± 5 mL of TSB containing 10% NaCl and 1% sodium pyruvate and incubated for 48 ± 2 h at 35–37°C.

Following enrichment, all test portions were streaked to BP agar plates. All plates were incubated for 48 h at 35–37°C. After incubation plates were examined for typical *S. aureus* colonies. Suspect typical colonies were transferred to a small tube containing 0.2–0.3 mL of BHI broth. A loopful of the BHI suspension was streaked to a TSA slant. Both BHI tubes and TSA slants were incubated for 18–24 h at 35–37°C. Following incubation, TSA slants were retained in the event of repeat testing. A 0.5 mL aliquot of reconstituted coagulase plasma with ethylenediaminetetraacetic acid (EDTA) was add to the BHI culture tube, mixed thoroughly, and incubated for 6 h at 35–37°C. The tube was examined periodically over the 6 h incubation for clot formation. Any sample with clots was further analyzed by Gram Stain. Those samples that were coagulase positive and contained Gram positive cocci were reported as positive.

ISO 6888-3:2003 method.—For the ISO 6888-3:2003 reference method, the 10 g test portions were enriched with 90 ± 5 mL of single-strength modified Giolitti-Cantoni broth. An additional 100 ± 5 mL of double-strength modified Giolitti-Cantoni broth was added. A plug of parafilm was then placed on top of the media. Samples were incubated for 24 ± 2 h at 37 ± 1°C. If blackening or black precipitate was present the sample was subcultured by streaking to BP agar plates. If no blackening or black precipitate was present the sample was incubated for an additional 24 ± 2 h at 37 ± 1°C before streaking to BP agar plates.

BP agar plates were incubated for 24 ± 2 h at 37 ± 1°C. Following incubation plates were examined and if no growth was present plates were incubated for an additional 24 ± 2 h at 37 ± 1°C. Plates were examined for typical *S. aureus* colonies. Suspect typical colonies were transferred to BHI broth and incubated for 24 ± 2 h at 37 ± 1°C. following incubation, a 0.1 mL aliquot of the BHI culture was transferred to 0.3 mL of rabbit plasma. Tubes were mixed thoroughly and incubated for up to 6 h at 37 ± 1°C. The tubes were examined periodically over 4–6 h of incubation for clot formation. If no clot formation was present tubes were re-incubated for an additional 24 h. Samples with clot formation present were reported as positive.

The Thermo Scientific SureTect Staphylococcus aureus PCR assay.—All test portions were prepared and incubated according to the protocol described previously in "*Sample Preparation*." All samples were analyzed by the Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay at 22 h and, regardless of presumptive results, were confirmed using the modified FDA/BAM Chapter 12 and ISO 6888-3:2003 reference method and the alternative confirmation as outlined in the "*Confirmation*" section.

As per criteria outlined in Appendix J of the *Official Methods of Analysis* Manual, fractional positive results were obtained for 100 g test portions of whey protein concentrate, whole milk powder, powdered infant formula, mozzarella cheese, and Edam cheese for the Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay. Prior to inoculation, an APC result of 2.8×10^2 CFU/g was obtained from the whey protein concentrate, 3.6×10^2 CFU/g was obtained for whole milk powder, 4.2×10^3CFU/g was obtained for powdered infant formula, 3.6×10^3 CFU/g was obtained from mozzarella cheese, and 2.2×10^2 CFU/g was obtained for Edam cheese. The percentage injury for the *S. aureus* ATCC 29738 used to inoculate the mozzarella cheese was 78.37%, and 78.57% for the *S. aureus* ATCC 33862 used to inoculate the Edam cheese. The POD was calculated as the number of positive outcomes divided by the total number of trials (11). The POD was calculated for the candidate presumptive results (POD_{CP}), the candidate confirmatory results (POD_{CC}), the difference in the candidate presumptive and confirmatory results ($dPOD_{CP}$), presumptive candidate results that confirmed positive (POD_C), the reference method (POD_R), and the difference in the confirmed candidate and reference methods ($dPOD_C$). The POD analysis between the Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay and the reference methods indicated that there was no significant difference at the 5% level between the number of positive results by the methods. The POD analysis between the Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay presumptive and confirmed results indicated that there was no significant difference at the 5% level for all methods following both the traditional confirmation procedure and the alternative confirmation procedure. A summary of POD analyses is presented in Tables 5–8 (12).

Robustness study.—For the robustness study the Thermo Scientific SureTect *Staphylococcus aureus* Assay was evaluated by adjusting three testing parameters. The three varied parameters included: enrichment time (21, 22, and 30 h), volume of sample lysed (9, 10, and 11 µL), and volume of lysate analyzed (18, 20, and 22 µL). A total of nine combinations of varied parameters were evaluated, with the ninth combination prepared as nominal conditions to provide a comparison. A total of twenty 100 g test portions of whey protein concentrate were analyzed. Ten were inoculated with *S. aureus* at a level to yield fractional positive results and ten were uninoculated. All 100 g test portions of whey protein concentrate were enriched with 900 ± 10 mL of Giolitti-Cantoni Broth modified with Potassium Tellurite and Tween 80 and incubated according to the enrichment times summarized in Table 9. All samples were randomized and tested in a blind-coded fashion.

The Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay demonstrated that small changes in testing parameters did not impact the performance of the assay. The POD results and 95% confidence intervals for each target analyte and treatment combination are presented in Table 10. The alternative confirmation procedure correctly detected the presence of *S. aureus* in all positive samples.

Results and Discussion

The Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay successfully detected *S. aureus* in 100 g whey protein concentrate, 100 g whole milk powder, 100 g powdered infant formula, 100 g mozzarella, and 100 g Edam cheese after 22 h. The

Table 5. Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay, candidate versus modified FDA/BAM Chapter 12 reference—POD results

				Candidate[c]			Reference				
Matrix	Strain	MPN/ test portion[a]	N[b]	x[d]	POD_C[e]	95% CI	x	POD_R[f]	95% CI	$dPOD_C$[g]	95% CI[h]
Whey protein concentrate (100 g)	*Staphylococcus aureus* QL030911-4[i]	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	−0.28, 0.38
		1.28 (0.63, 2.61)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Whole milk powder (100 g)	*Staphylococcus aureus* ATCC 11632[k]	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.49 (0.25, 0.84)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		1.28 (0.63, 2.61)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Powdered infant formula (100 g)	*Staphylococcus aureus* ATCC 25923	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.49 (0.25, 0.84)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	−0.19, 0.37
		1.65 (0.80, 3.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Mozzarella cheese (100 g)	*Staphylococcus aureus* ATCC 29737	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.65 (0.36, 1.10)	20	8	0.40	0.22, 0.61	9	0.45	0.26, 0.66	−0.05	−0.33, 0.24
		1.51 (0.75, 3.05)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Edam cheese (100 g)	*Staphylococcus aureus* ATCC 33862	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.47 (0.24, 0.81)	20	10	0.50	0.30, 0.70	8	0.40	0.22, 0.61	0.10	−0.19, 0.37
		1.08 (0.52, 2.24)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43

[a] MPN = Most probable number, calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b] N = Number of test portions.
[c] Results were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real-Time PCR Instrument.
[d] x = Number of positive test portions.
[e] POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g] $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] QL = Q Laboratories Culture Collection, Cincinnati, OH.
[j] N/A = Not applicable.
[k] ATCC = American Type Culture Collection, Manassas, VA.

Table 6. Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay, candidate versus ISO 6888-3:2003 reference—POD results

				Candidate[c]			Reference				
Matrix	Strain	MPN/ test portion[a]	N[b]	X[d]	POD_C[e]	95% CI	X	POD_R[f]	95% CI	$dPOD_C$[g]	95% CI[h]
Whey protein concentrate (100 g)	*Staphylococcus aureus* QL030911-4[i]	N/A[j]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.81 (0.48, 1.33)	20	7	0.35	0.18, 0.57	10	0.50	0.30, 0.70	−0.15	−0.41, 0.15
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Whole milk powder (100 g)	*Staphylococcus aureus* ATCC 11632[k]	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.42 (0.21, 0.74)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Powdered infant formula (100 g)	*Staphylococcus aureus* ATCC 25923	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.42 (0.21, 0.74)	20	10	0.50	0.30, 0.70	7	0.35	0.18, 0.57	0.15	−0.15, 0.41
		1.25 (0.59, 2.67)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Mozzarella cheese (100 g)	*Staphylococcus aureus* ATCC 29737	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.49 (0.25, 0.84)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43
Edam cheese (100 g)	*Staphylococcus aureus* ATCC 33862	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.43, 0.43
		0.65 (0.37, 1.08)	20	10	0.50	0.30, 0.70	9	0.45	0.26, 0.66	0.05	−0.24, 0.33
		1.48 (0.71, 3.10)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.43, 0.43

[a] MPN = Most probable number, calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b] N = Number of test portions.
[c] Results were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real-Time PCR Instrument.
[d] x = Number of positive test portions.
[e] POD_C = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.
[f] POD_R = Reference method confirmed positive outcomes divided by the total number of trials.
[g] $dPOD_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values.
[h] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[i] QL = Q Laboratories Culture Collection, Cincinnati, OH.
[j] N/A = Not applicable.
[k] ATCC = American Type Culture Collection, Manassas, VA.

Table 7. Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay, presumptive versus confirmed—FDA/BAM Chapter 12 POD results

Matrix	Strain	MPN/ test portion[a]	N[b]	Presumptive[c]			Confirmed[f]				
				x[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[g]	95% CI	$dPOD_{CP}$[h]	95% CI[i]
Whey protein concentrate (100 g)	*Staphylococcus aureus* QL030911-4[j]	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.49 (0.25, 0.84)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	−0.13, 0.13
		1.28 (0.63, 2.61)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Whole milk powder (100 g)	*Staphylococcus aureus* ATCC11632[l]	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.49 (0.25, 0.84)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	−0.13, 0.13
		1.28 (0.63, 2.61)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Powdered infant formula (100 g)	*Staphylococcus aureus* ATCC 25923	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.49 (0.25, 0.84)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	−0.13, 0.13
		1.65 (0.80, 3.40)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Mozzarella cheese (100 g)	*Staphylococcus aureus* ATCC 29737	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.65 (0.36, 1.10)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	−0.13, 0.13
		1.51 (0.75, 3.05)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Edam cheese (100 g)	*Staphylococcus aureus* ATCC 33862	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.47 (0.24, 0.81)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	−0.13, 0.13
		1.08 (0.52, 2.24)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47

[a] MPN = Most probable number, calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b] N = Number of test portions.
[c] Results were identical for analysis conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and 7500 Fast Real—Time PCR Instrument.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] Results obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Chapter 12 reference method.
[g] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] $dPOD_{CP}$= Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[i] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j] QL = Q Laboratories Culture Collection, Cincinnati, OH.
[k] N/A = Not applicable.
[l] ATCC = American Type Culture Collection, Manassas, VA.

Table 8. Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay, presumptive versus confirmed—ISO 6888-3:2003 POD results

Matrix	Strain	MPN/ test portion[a]	N[b]	Presumptive[c]			Confirmed[f]				
				x[d]	POD_{CP}[e]	95% CI	x	POD_{CC}[g]	95% CI	$dPOD_{CP}$[h]	95% CI[i]
Whey protein concentrate (100 g)	*Staphylococcus aureus* QL030911–4[j]	N/A[k]	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.81 (0.48, 1.33)	20	7	0.35	0.18, 0.57	7	0.35	0.18, 0.57	0.00	−0.13, 0.13
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Whole milk powder (100 g)	*Staphylococcus aureus* ATCC11632[l]	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.42 (0.21, 0.74)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	−0.13, 0.13
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Powdered infant formula (100 g)	*Staphylococcus aureus* ATCC 25923	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.42 (0.21, 0.74)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	−0.13, 0.13
		1.25 (0.59, 2.67)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Mozzarella cheese (100 g)	*Staphylococcus aureus* ATCC 29737	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.49 (0.25, 0.84)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	−0.13, 0.13
		2.02 (0.93, 4.39)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47
Edam cheese (100 g)	*Staphylococcus aureus* ATCC 33862	N/A	5	0	0.00	0.00, 0.43	0	0.00	0.00, 0.43	0.00	−0.47, 0.47
		0.65 (0.37, 1.08)	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0.00	−0.13, 0.13
		1.48 (0.71, 3.10)	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	−0.47, 0.47

[a] MPN = Most probable number, calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.
[b] N = Number of test portions.
[c] Results were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real-Time PCR Instrument.
[d] x = Number of positive test portions.
[e] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[f] Results obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Chapter 12 reference method.
[g] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[h] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[i] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j] QL = Q Laboratories Culture Collection, Cincinnati, OH.
[k] N/A = Not applicable.
[l] ATCC = American Type Culture Collection, Manassas, VA.

candidate method tested 100 g portion sizes compared to 10 g portion sizes for the ISO reference method and 50 g portion sizes for the modified FDA/BAM (used as a detection method) reference method. POD analysis of the data showed no statistically significant differences between the candidate method or either of the reference methods, demonstrating high sensitivity and robust performance of the candidate method considering the differing sample sizes but uniform spiking levels. POD analysis also demonstrated no statistically significant differences between presumptive positives and confirmed positives for the candidate method.

Table 9. Combinations evaluated in robustness study

Combination	Enrichment time, h	Sample volume lysed, μL	Volume of lysate analyzed, μL
1	21	9	18
2	21	9	22
3	21	11	18
4	21	11	22
5	30	9	18
6	30	9	22
7	30	11	18
8	30	11	22
9	22	10	20

The inclusivity/exclusivity study correctly identified all 50 inclusivity isolates tested and excluded all of the 52 exclusivity isolates tested, highlighting the specificity of the method.

The stability study results, and consequential POD analysis, demonstrated no significant differences between kit lots, showing that manufacturing and performance are equivalent between kit lots demonstrating no overall degradation of the product over time, supporting the shelf life statement.

The results of the robustness study showed equivalent performance between the test and nominal conditions. POD analysis showed no statistically significant differences between the nominal and test conditions at the 5% confidence level, demonstrating that typical small parameter deviations that might occur when performed by an end user do not impact assay performance.

The Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay is robust, quick, and simple to perform, providing results in around 80 min post enrichment. The Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real-Time PCR Instrument software is user friendly and easy to use.

Conclusions

The data presented in this report, within their statistical uncertainty, support the product claims that the Thermo Scientific SureTect *Staphylococcus aureus* PCR workflow constitutes a rapid and reliable workflow for the detection of *S. aureus* in dairy matrixes. The results obtained by the POD analysis of the matrix

Table 10. Robustness Thermo Scientific SureTect *Staphylococcus aureus* PCR Assay—POD results

	Test parameters				Test condition results[c]			Nominal condition results[c,f]				
Test combination[a]	Enrichment time, h	Sample volume lysed, μL	Volume of lysate analyzed, μL	N[b]	x[d]	POD_T[e]	95% CI	x	POD_N[g]	95% CI	$dPOD_{TN}$[h]	95% CI[i]
Inoculated test portions whey protein concentrate (*Staphylococcus aureus* QL030911–4)[j]												
1	21	9	18	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
2	21	9	22	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
3	21	11	18	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
4	21	11	22	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
5	30	9	18	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
6	30	9	22	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
7	30	11	18	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
8	30	11	22	10	7	0.70	0.40, 0.89	7	0.70	0.40, 0.89	0.00	−0.36, 0.36
Uninoculated test portions whey protein concentrate												
1	21	9	18	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
2	21	9	22	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
3	21	11	18	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
4	21	11	22	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
5	30	9	18	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
6	30	9	22	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
7	30	11	18	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28
8	30	11	22	10	0	0.00	0.00, 0.28	0	0.00	0.00, 0.28	0.00	−0.28, 0.28

[a] Each test condition is being compared to the nominal test condition. *Note*: Test conditions 1–4 (21 h enrichment) and test conditions 5–8 (30 h enrichment) were compared to the nominal condition in different experiments.
[b] N = Number of test portions per condition.
[c] Results were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real-Time PCR Instrument.
[d] x = Number of positive test portions per condition.
[e] POD_T = Positive outcomes divided by the total number of trials per condition.
[f] Nominal condition = 22 Hours enrichment, 10 μL of sample volume lysed, and 20 μL of lysate analyzed.
[g] POD_N = Positive outcomes divided by the total number of trials per nominal condition.
[h] $dPOD_{TN}$ = Difference in POD between the test condition and nominal condition.
[i] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[j] QL = Q Laboratories Culture Collection, Cincinnati, OH.

study demonstrated that there were no statistically significant differences between the number of positive samples detected by the candidate and the reference methods for all samples tested. The inclusivity/exclusivity study demonstrated 100% specificity of the method. The product consistency and stability study demonstrated no difference in assay performance in kit lots at different expiry time points and the robustness study demonstrated that small deviations in testing parameters did not impact the performance of the assay. In addition, no statistically significant differences were observed between the alternative confirmation and the traditional reference method confirmation.

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Conflict of Interest

There are no conflicts of interest from any involved author.

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