



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
052101

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay

manufactured by

Oxoid Ltd. part of Thermo Fisher Scientific
Wade Road
Basingstoke
Hampshire, RG24 8PW

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

October 10, 2024
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METHOD NAME Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay	CATALOG NUMBER A56839
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INDEPENDENT LABORATORY Q Laboratories, Inc. 1400 Harrison Avenue Cincinnati, OH 45214 USA	APPLICABILITY OF METHOD Target Organisms – <i>Staphylococcus aureus</i> . Matrixes – (up to 100 g portions) - Whey protein concentrate, whole milk powder, probiotic powdered infant formula, edam cheese and mozzarella cheese. Performance claims – Method not statistically different compared to the ISO 6888-3:2003 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (<i>Staphylococcus aureus</i> and other species) – Part 3: Detection and MPN technique for low numbers after probability of detection (POD) analysis (2) and the U. S. Food and Drug Administration Bacteriological Analytical Manual, Chapter 12 (2016), <i>Staphylococcus aureus</i> (FDA/BAM Chapter 12) (3) reference methods.
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ORIGINAL CERTIFICATION DATE May 07, 2021	CERTIFICATION RENEWAL RECORD Renewed annually through December 2025.
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METHOD MODIFICATION RECORD 1. July 2022 Level 2 2. December 2022 Level 1 3. January 2024 Level 1 4. January 2024 Level 2	SUMMARY OF MODIFICATION 1. Changes made to improve handling steps and visual indicators. 2. Editorial/clerical changes. 3. Editorial/clerical changes. 4. Addition of automated lysis procedure and PCR setup procedure.
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PRINCIPLE OF THE METHOD (1)

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 *Staphylococcus aureus* from dairy samples.

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 *Staphylococcus 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 minutes 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. The candidate confirmation procedure allows for rapid confirmation of *Staphylococcus aureus* isolates through sample culture onto Brilliance™ Staph 24 agar or Baird-parker Agar, with consequential biochemical confirmation of isolated characteristic colonies achieved within minutes by the Thermo Scientific Staphaurex™ Latex Agglutination Test.

DISCUSSION OF THE VALIDATION STUDY (1)

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 hours. The 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.

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 minutes 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.

Table 2: Inclusivity results of Thermo Scientific SureTect Staphylococcus aureus PCR Assay. (1)

Inclusivity results				
ID	Source	Origin	Strain name	SureTect <i>S. aureus</i>
				Result
ATCC ^a	33591	Human - Clinical	<i>Staphylococcus aureus</i>	+
RDCC ^b	0069	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	0070	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	0071	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	122	Unknown	<i>Staphylococcus aureus</i>	+
ATCC	9144	Human - Clinical	<i>Staphylococcus aureus</i>	+
RDCC	254	Unknown	<i>Staphylococcus aureus</i>	+
ATCC	12600	Pleural Fluid	<i>Staphylococcus aureus</i>	+
ATCC	6538	Human Lesion	<i>Staphylococcus aureus</i>	+
RDCC	502	Unknown	<i>Staphylococcus aureus</i>	+
ATCC	29737	Unknown	<i>Staphylococcus aureus</i>	+
NCTC ^c	7447	Unknown	<i>Staphylococcus aureus</i>	+
NCTC	13143	Unknown	<i>Staphylococcus aureus</i>	+
ATCC	43300	Human - Clinical, Kansas	<i>Staphylococcus aureus</i>	+
NCTC	10442	Human - Finger	<i>Staphylococcus aureus</i>	+
NCTC	12493	Human - Clinical	<i>Staphylococcus aureus</i>	+
RDCC	900	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	922	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	923	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	930	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	931	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	1105	Germany - Human	<i>Staphylococcus aureus</i>	+
RDCC	1106	Germany - Human	<i>Staphylococcus aureus</i>	+
RDCC	1645	Unknown	<i>Staphylococcus aureus</i>	+
ATCC	49476	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	2953	Food Res Inst of Wisconsin	<i>Staphylococcus aureus</i>	+
RDCC	3053	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	3901	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	3903	Denka - Japan	<i>Staphylococcus aureus</i>	+
RDCC	3904	Oxoid, France	<i>Staphylococcus aureus</i>	+
RDCC	3917	ex UCH	<i>Staphylococcus aureus</i>	+
RDCC	3918	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	3919	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	4011	Alfred Hosp, Melbourne - Human	<i>Staphylococcus aureus</i>	+
RDCC	4012	Alfred Hosp, Melbourne - Human	<i>Staphylococcus aureus</i>	+
RDCC	4658	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	4659	Unknown	<i>Staphylococcus aureus</i>	+
RDCC	4761	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	4762	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	4763	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	4764	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	4765	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	5058	US - Wound Swab (Human)	<i>Staphylococcus aureus</i>	+
RDCC	5059	US - Wound Swab (Human)	<i>Staphylococcus aureus</i>	+
RDCC	5060	US - Wound Swab (Human)	<i>Staphylococcus aureus</i>	+
RDCC	5145	US - Clinical Swab	<i>Staphylococcus aureus</i>	+
RDCC	5268	Unknown	<i>Staphylococcus aureus</i>	+
NCTC	12497	Clinical	<i>Staphylococcus aureus</i>	+
NCTC	10345	Clinical	<i>Staphylococcus aureus</i>	+
RDCC	482	Unknown	<i>Staphylococcus aureus</i>	+

^aATCC = American Type Culture Collection, Manassas, VA, USA.

^bRDCC = Thermo Fisher Scientific, Research and Development Culture Collection, Basingstoke, UK.

^aNCTC = National Collection of Type Cultures, Salisbury, UK.

Table 3: Exclusivity results of Thermo Scientific SureTect Staphylococcus aureus PCR Assay. (1)

Exclusivity results				
ID	Source	Origin	Strain name	SureTect <i>S. aureus</i>
				Result
TCC ^a	627	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	629	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	632	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	633	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	634	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	635	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	636	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	637	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	638	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	639	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	640	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	641	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	642	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	643	Germany	Coagulase Negative <i>Staphylococcus</i>	-
TCC	644	Germany	Coagulase Negative <i>Staphylococcus</i>	-
ATCC ^b	27853	Blood - Human	<i>Pseudomonas aeruginosa</i>	-
ATCC	13525	Pre-Filter Water Tanks, England	<i>Pseudomonas fluorescens</i>	-
RDCC ^c	2170	Unknown	<i>Pseudomonas putida</i>	-
ATCC	4973	Unknown	<i>Pseudomonas fragi</i>	-
RDCC	2977	Unknown	<i>Pseudomonas stutzeri</i>	-
RDCC	2980	Unknown	<i>Pseudomonas vesicularis</i>	-
RDCC	1415	Unknown	<i>Staphylococcus capitis</i>	-
RDCC	1789	Unknown	<i>Staphylococcus caprae</i>	-
ATCC	51365	Dry sausage	<i>Staphylococcus carnosus</i>	-
RDCC	1931	Grenoble, France	<i>Staphylococcus chromogenes</i>	-
RDCC	2812	King Georges hospital, Redbridge	<i>Staphylococcus cohnii</i>	-
RDCC	669	Human (hospital)	<i>Staphylococcus epidermidis</i>	-
RDCC	670	Human (hospital)	<i>Staphylococcus epidermidis</i>	-
RDCC	1319	Unknown	<i>Staphylococcus haemolyticus</i>	-
RDCC	1320	Unknown	<i>Staphylococcus haemolyticus</i>	-
RDCC	986	Unknown	<i>Staphylococcus hominis</i>	-
RDCC	1162	Unknown	<i>Staphylococcus hominis</i>	-
RDCC	2695	Hospital Edouard Herriot, Lyon - Human	<i>Staphylococcus hyicus</i>	-
RDCC	121	Human	<i>Staphylococcus intermedius</i>	-
RDCC	2897	Salmon	<i>Staphylococcus lentus</i>	-
RDCC	5621	Germany - culture media contaminant	<i>Staphylococcus lentus</i>	-
NCTC ^d	7990	Blood Culture	<i>Staphylococcus lugdenensis</i>	-
ATCC	43809	Auxillary Lymph Node, France	<i>Staphylococcus lugdenensis</i>	-
RDCC	550	Vetinary Isolate	<i>Staphylococcus pseudointermedius</i>	-
RDCC	551	Vetinary Isolate	<i>Staphylococcus pseudointermedius</i>	-
ATCC	15305	Urine - Human	<i>Staphylococcus saprophyticus</i>	-
RDCC	1933	Limoges Hospital - Human	<i>Staphylococcus schleiferi</i>	-
RDCC	2680	Hospital Edouard Herriot, Lyon - Human	<i>Staphylococcus schleiferi</i>	-
RDCC	4379	Unknown	<i>Staphylococcus sciuri</i>	-
RDCC	4069	Basildon Hospital - Human	<i>Staphylococcus simulans</i>	-
RDCC	1930	Unknown	<i>Staphylococcus simulans</i>	-
RDCC	1321	Unknown	<i>Staphylococcus warneri</i>	-
RDCC	1429	Unknown	<i>Staphylococcus warneri</i>	-
ATCC	29971	Human skin	<i>Staphylococcus xylosus</i>	-
RDCC	3748	Unknown	<i>Staphylococcus xylosus</i>	-
RDCC	2972	Unknown	<i>Stenotrophomonas maltophilia</i>	-

^aTCC = Internal Trials Culture Collection, Thermo Fisher Scientific, Basingstoke, UK.

^bATCC = American Type Culture Collection, Manassas, VA, USA.

^cRDCC = Thermo Fisher Scientific, Research and Development Culture Collection, Basingstoke, UK.

^dNCTC = National Collection of Type Cultures, Salisbury, UK.

Table 4. Thermo Scientific SureTect Staphylococcus aureus PCR Assay, Candidate vs. modified FDA/BAM Chapter 12 Reference – POD Results. (1)

Matrix	Strain	MPN ^a / Test Portion	N ^b	Candidate ^c			Reference			dPOD _c ^g	95% CI ^h
				X ^d	POD _c ^e	95% CI	X	POD _R ^f	95% CI		
Whey Protein Concentrate (100 g)	<i>Staphylococcus aureus</i> QL'030911-4	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	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)	<i>Staphylococcus aureus</i> ATCC ^k 11632	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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cResults were identical for analysis conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.

^dX = Number of positive test portions.

^ePOD_c = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

^fPOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱQL = Q Laboratories Culture Collection, Cincinnati, OH.

^jN/A = Not applicable.

^kATCC = American Type Culture Collection, Manassas, VA.

Table 5. Thermo Scientific SureTect Staphylococcus aureus PCR Assay, Candidate vs. ISO 6888-3:2003 Reference – POD Results. (1)

Matrix	Strain	MPN ^a / Test Portion	N ^b	Candidate ^c			Reference			dPOD _c ^g	95% CI ^h
				X ^d	POD _c ^e	95% CI	X	POD _R ^f	95% CI		
Whey Protein Concentrate (100 g)	<i>Staphylococcus aureus</i> QL'030911-4	N/A	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)	<i>Staphylococcus aureus</i> ATCC ^k 11632	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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval.

^bN = Number of test portions.

^cResults were identical for analysis conducted on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.

^dX = Number of positive test portions.

^ePOD_c = Candidate method presumptive positive outcomes confirmed positive divided by the total number of trials.

^fPOD_R = Reference method confirmed positive outcomes divided by the total number of trials.

^gdPOD_c = Difference between the confirmed candidate method result and reference method confirmed result POD values.

^h95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱQL = Q Laboratories Culture Collection, Cincinnati, OH.

^jN/A = Not applicable.

^kATCC = American Type Culture Collection, Manassas, VA.

Table 6. Thermo Scientific SureTect Staphylococcus aureus PCR Assay, Presumptive vs. Confirmed–FDA/BAM Chapter 12 POD Results. (1)

Matrix	Strain	MPN ^a / Test Portion	N ^b	Presumptive ^c			Confirmed ^f			dPOD _{CP} ^h	95% CI ⁱ
				X ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^g	95% CI		
Whey Protein Concentrate (100 g)	<i>Staphylococcus aureus</i> QL/030911-4	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)	<i>Staphylococcus aureus</i> ATCC 11632	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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cResults were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.

^dX = Number of positive test portions

^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^fResults obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Chapter 12 reference method.

^gPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^hdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

ⁱ95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^kQL = Q Laboratories Culture Collection, Cincinnati, OH.

^lN/A = Not applicable.

^mATCC = American Type Culture Collection, Manassas, VA.

Table 7. Thermo Scientific SureTect Staphylococcus aureus PCR Assay, Presumptive vs. Confirmed–ISO 6888-3:2003 POD Results. (1)

Matrix	Strain	MPN ^a / Test Portion	N ^b	Presumptive ^c			Confirmed ^f			dPOD _{CP} ^h	95% CI ⁱ
				X ^d	POD _{CP} ^e	95% CI	X	POD _{CC} ^g	95% CI		
Whey Protein Concentrate (100 g)	<i>Staphylococcus aureus</i> QL/030911-4	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)	<i>Staphylococcus aureus</i> ATCC 11632	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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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)	<i>Staphylococcus aureus</i> 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

^aMPN = Most Probable Number is calculated using the LCF MPN calculator ver. 1.6 provided by AOAC RI, with 95% confidence interval

^bN = Number of test portions

^cResults were identical for analysis conducted on the Applied Biosystems QuantStudio 5 Real-Time PCR instrument and 7500 Fast Real – Time PCR Instrument.

^dX = Number of positive test portions

^ePOD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials

^fResults obtained following the alternative confirmation were identical to results obtain from confirmation by FDA/BAM Chapter 12 reference method.

^gPOD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials

^hdPOD_{CP} = Difference between the candidate method presumptive result and candidate method confirmed result POD values

ⁱ95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level

^kQL = Q Laboratories Culture Collection, Cincinnati, OH.

^lN/A = Not applicable.

^mATCC = American Type Culture Collection, Manassas, VA.

DISCUSSION OF THE MODIFICATION STUDY APPROVED JANUARY 2024 (4)

The comparison study was selected to evaluate the automated procedure as it allowed for an accurate and precise comparison of the performance between the manual and automated lysis and PCR setup procedures without interference from other parts of the method, such as the enrichment. The study followed a paired study design with a post enrichment spike to assess the performance of the lysis and PCR setup procedures specifically.

Comparison studies above the LOD of the PCR assays showed that the difference in average C_t values were always ±1.5 cycles when comparing the automated and manual procedures. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual.

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