AOAC *PERFORMANCE TESTED METHODS*℠ NEWS

Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay Granted PTM Status

Thermo Scientific™

Test Kit: SureTect™ Staphylococcus aureus PCR Assay

PTM Certificate No.: 052101

The Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay is a real-time PCR kit for the rapid detection of *Staphylococcus aureus* from dairy matrices (Figure 1). Results are obtained in 80 minutes post-enrichment, and the assay can be used in conjunction with the Applied Biosystems™ 7500 Fast Real-Time PCR instrument and Applied Biosystems™ QuantStudio™ 5 Real-Time PCR instrument.

Figure 1. Thermo Scientific™ SureTect™ Staphylococcus aureus PCR Assay is a real-time PCR kit for the rapid detection of Staphylococcus aureus from dairy matrices.

The assay is based on TaqMan™ PCR technology and 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-labeled probes, and PCR master mix components) to easily conduct PCR analysis of the sample.

The Thermo Scientific Staphylococcus aureus PCR Assay was evaluated for AOAC® *Performance Tested Methods*℠ (PTM) certification according to AOAC guidelines for validation of microbiological methods following an unpaired study design.

Table 1. Thermo Scientific Staphylococcus aureus PCR Assay results, candidate vs modified FDA/BAM Chapter 12 reference—POD results

Matrix	Strain	Time point[a], h	Contamination level/area[b]	*N*[c]	Candidate	FDA/BAM Ch. 12	95% CI[e]
					X[d]	*X*[d]	
Whey protein concentrate (100 g)	*S. aureus* QL[f] 030911-4	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	7	7	–0.28, 0.38
			1.28 (0.63, 2.61)	5	5	5	–0.43, 0.43
Whole milk powder (100 g)	*S. aureus* ATCC[g] 11632	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	8	7	–0.23, 0.32
			1.28 (0.63, 2.61)	5	5	5	–0.43, 0.43
Powdered infant formula with probiotics (100 g)	*S. aureus* ATCC 25923	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	10	8	–0.19, 0.37
			1.65 (0.80, 3.40)	5	5	5	–0.43, 0.43
Mozzarella cheese (100 g)	*S. aureus* ATCC 29737	22	N/A	5	0	0	–0.43, 0.43
			0.65 (0.36, 1.10)	20	8	9	–0.33, 0.24
			1.51 (0.75, 3.05)	5	5	5	–0.43, 0.43
Edam cheese (100 g)	*S. aureus* ATCC 33862	22	N/A	5	0	0	–0.43, 0.43
			0.47 (0.24, 0.81)	20	10	8	–0.19, 0.37
			1.08 (0.52, 2.24)	5	5	5	–0.43, 0.43

[a] Results were identical at 22 hours for both PCR instruments.
[b] Contamination level = CFU level/sample.
[c] *N* = Number of test portions.
[d] *X* = Number of positive test portions.
[e] 95% CI = POD confidence interval. If the two numbers do not sit either side of '0.00,' then the difference is statistically significant at the 5% level.
[f] QL = Q Laboratories Culture Collection, Cincinnati, Ohio, USA.
[g] ATCC = American Type Culture Collection, Manassas, Virginia, USA.

Method developer studies were conducted in the laboratories of Thermo Fisher Scientific, Basingstoke, UK, and Vantaa, Finland, and included the inclusivity/exclusivity and lot-to-lot product consistency and stability studies.

The independent laboratory study was conducted by Q Laboratories, Cincinnati, Ohio, USA, and comprised matrix and robustness studies. The matrix study consisted of testing 100 g whey protein concentrate, 100 g whole milk powder, 100 g powdered infant formula (with probiotics), 100 g mozzarella, and 100 g Edam cheese. All matrices were tested against 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 a modified version of the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (FDA/BAM) Chapter 12, *Staphylococcus aureus,* 2016, reference methods.

The FDA/BAM reference method was modified from the most probable number (MPN) format to a detection format to allow for comparison to the candidate nonquantitative detection method. As MPN methods are based on a series of qualitative methods, it was determined that using the qualitative method in the FDA/BAM MPN for *S. aureus* 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. This modification allowed for direct comparison of the candidate qualitative method by altering the FDA reference methodology to detection but adhering to determined enrichment procedures and ratios.

The inclusivity/exclusivity study examined 50 *S. aureus* inclusivity isolates and 51 exclusivity isolates, which comprised several nontarget *Staphylococcus* strains and closely related bacterial species on both the QuantStudio 5 and 7500 Fast instruments. Inclusivity isolates were cultured in Giolitti-Cantoni Broth, and exclusivity isolates were cultured in nonselective enrichment broth. The method showed 100% specificity with all inclusivity isolates correctly identified, but no exclusivity isolates detected.

The product consistency and lot-to-lot stability study examined three kit lots at different stages of expiry within the stipulated shelf life. The three kits tested were newly manufactured (fresh—1 month old), middle of the shelf life (mid—7 months old), and near the end of shelf life (end—11 months old). An *S. aureus* strain was selected for use as a target organism to compare the performance of the kits at different expiry stages. A strain of *Staphylococcus epidermidis* was selected as a nontarget organism for comparison between the three kits, specifically to check for false-positive results. Data from the study was ana-

Table 2. Thermo Scientific Staphylococcus aureus PCR Assay results, candidate vs ISO 6888-3:2003 reference—POD results

Matrix	Strain	Time point[a], h	Contamination level/area[b]	*N*[c]	Candidate	ISO 6888-3:2003	95% CI[e]
					X[d]	*X*[d]	
Whey protein concentrate (100 g)	*S. aureus* QL[f] 030911-4	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	7	10	–0.28, 0.38
			1.28 (0.63, 2.61)	5	5	5	–0.43, 0.43
Whole milk powder (100 g)	*S. aureus* ATCC[g] 11632	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	8	7	–0.23, 0.32
			1.28 (0.63, 2.61)	5	5	5	–0.43, 0.43
Powdered infant formula with probiotics (100 g)	*S. aureus* ATCC 25923	22	N/A	5	0	0	–0.43, 0.43
			0.49 (0.25, 0.84)	20	10	7	–0.19, 0.37
			1.65 (0.80, 3.40)	5	5	5	–0.43, 0.43
Mozzarella cheese (100 g)	*S. aureus* ATCC 29737	22	N/A	5	0	0	–0.43, 0.43
			0.65 (0.36, 1.10)	20	8	7	–0.33, 0.24
			1.51 (0.75, 3.05)	5	5	5	–0.43, 0.43
Edam cheese (100 g)	*S. aureus* ATCC 33862	22	N/A	5	0	0	–0.43, 0.43
			0.47 (0.24, 0.81)	20	10	9	–0.19, 0.37
			1.08 (0.52, 2.24)	5	5	5	–0.43, 0.43

[a] Results were identical at 22 hours for both PCR instruments.
[b] Contamination level = CFU level/sample.
[c] *N* = Number of test portions.
[d] *X* = Number of positive test portions.
[e] 95% CI = POD confidence interval. If the two numbers do not sit either side of '0.00,' then the difference is statistically significant at the 5% level.
[f] QL = Q Laboratories Culture Collection, Cincinnati, Ohio, USA.
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Table 3. Thermo Scientific Staphylococcus aureus PCR Assay results, presumptive vs confirmed—FDA/BAM Chapter 12 and ISO 6888-3:2003 POD results

Matrix	Strain	Time point[a], h	Contamination level/area[b]	*N*[c]	Candidate	Reference	95% CI[e]
					X[d]	*X*[d]	
Whey protein concentrate (100 g)	*S. aureus* QL[f] 030911-4	22	N/A	5	0	0	–0.47, 0.47
			0.49 (0.25, 0.84)	20	7	7	–0.13, 0.13
			1.28 (0.63, 2.61)	5	5	5	–0.47, 0.47
Whole milk powder (100 g)	*S. aureus* ATCC[g] 11632	22	N/A	5	0	0	–0.47, 0.47
			0.49 (0.25, 0.84)	20	8	8	–0.13, 0.13
			1.28 (0.63, 2.61)	5	5	5	–0.47, 0.47
Powdered infant formula with probiotics (100 g)	*S. aureus* ATCC 25923	22	N/A	5	0	0	–0.47, 0.47
			0.49 (0.25, 0.84)	20	10	10	–0.13, 0.13
			1.65 (0.80, 3.40)	5	5	5	–0.47, 0.47
Mozzarella cheese (100 g)	*S. aureus* ATCC 29737	22	N/A	5	0	0	–0.47, 0.47
			0.65 (0.36, 1.10)	20	8	8	–0.13, 0.13
			1.51 (0.75, 3.05)	5	5	5	–0.47, 0.47
Edam cheese (100 g)	*S. aureus* ATCC 33862	22	N/A	5	0	0	–0.47, 0.47
			0.47 (0.24, 0.81)	20	10	10	–0.13, 0.13
			1.08 (0.52, 2.24)	5	5	5	–0.47, 0.47

[a] Results were identical at 22 hours for both PCR instruments.
[b] Contamination level = CFU level/sample.
[c] *N* = Number of test portions.
[d] *X* = Number of positive test portions.
[e] 95% CI = POD confidence interval. If the two numbers do not sit either side of '0.00,' then the difference is statistically significant at the 5% level.
[f] QL = Q Laboratories Culture Collection, Cincinnati, Ohio, USA.
[g] ATCC = American Type Culture Collection, Manassas, Virginia, USA.

lyzed using probability of detection (POD), which showed that there were no statistically significant differences in performance between any of the kits lots, demonstrating kit consistency across the stipulated shelf life. There were no false positives or false negatives observed for any of the kits at any expiration point.

The robustness study evaluated potential impact on performance of the assay after set parameter deviations. Three parameter deviations were investigated that were most likely to occur when the workflow was performed by an end user: enrichment time, volume of sample lysed, and volume of lysate analyzed. A total of nine combinations were tested with the ninth combination prepared as nominal conditions to provide a comparison. Twenty 100 g test portions of whey protein concentrate were analyzed, 10 inoculated with a strain of *S. aureus* and 10 uninoculated. The data generated by the study was analyzed using POD, which showed that there were no statistically significant differences between any of the combinations tested or compared to the nominal conditions. The Thermo Scientific SureTect Staphylococcus aureus PCR Assay is robust with performance not impacted by method parameter deviations.

The matrix study conducted by the independent laboratory used samples artificially contaminated with *S. aureus* and tested a total of 30 replicates per matrix: five unspiked samples (US), 20 low-spiked samples (LS) to yield fractionally positive results, and five high-spiked samples (HS). All samples were analyzed at 22 hours of enrichment on both the QuantStudio 5 and 7500 Fast. Results for the matrix study are shown in Tables 1–3.

The matrix study data showed that there were no statistically significant differences between the candidate method and either reference method after POD analysis. Further, the candidate confirmation procedure allows for rapid confirmation of *S. aureus* isolates through culture onto Brilliance™ Staph 24 Agar or Baird-Parker Agar, with consequential biochemical confirmation of isolated colonies achieved within minutes by the Thermo Scientific™ Staphaurex™ latex agglutination test in as little as 24 hours compared to a minimum of 2 days for reference method techniques. In addition, there were also no statistically significant differences found between presumptive and confirmed positives for either reference method when analyzed by POD.

The data presented shows that the Thermo Scientific SureTect Staphylococcus aureus PCR Assay offers a rapid and reliable workflow for detection of *S. aureus* from dairy matrices. The assay is robust, stable across the stated shelf life, shows 100% specificity, and has comparable performance to both reference methods. The method was granted PTM status (Certificate No. 052101). ■