Development and Validation of Hygiena's Real-Time PCR Assay for the Detection and Identification of Coagulase-Positive *Staphylococcus* species and *S. aureus*

One Health Diagnostics™

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BAX® System Q7 foodproof®

INTRODUCTION:

Staphylococcus food poisoning is a gastrointestinal illness caused by foods contaminated with toxins produced predominantly by *Staphylococcus aureus* but sometimes also by other coagulase-positive staphylococci (CPS).

Many people and animals have coagulase-positive staphylococci present on their skin and nose or are intestinal carriers. These pathogens also cause highly contagious mastitis in dairy cows, one of the major contamination routes for raw milk.

Culture methods are laborious, time-consuming, and difficult to interpret to provide rapid results to identify and control the spread of infections.

SIGNIFICANCE:

Providing the ready-to-eat, dairy and other food service industries with a rapid, easy to use PCR method for combined detection of coagulase-positive staph and *Staphylococcus aureus* that meets all regulatory requirements and can significantly improve contamination management of this highly infectious pathogen.

PURPOSE:

The aim of these studies was to develop a rapid, real-time PCR assay that detects all coagulase-positive *Staphylococcus* species (CPS) and simultaneously identifies the most relevant CPS species, *Staphylococcus aureus*.
Fast time to results: DNA isolation & PCR < 2h
(with enrichment 24 ± 2h)

Manufacturer: BIOTECON Diagnostics GmbH
Hermannswerder 17
14473 Potsdam
GERMANY

foodproof® *Staphylococcus* Detection LyoKit®:

The **food**proof *Staphylococcus* Detection LyoKit provides all necessary reagents and a control template for reliable interpretation of results. All food-relevant, coagulase-positive *Staphylococcus* species are jointly detected in the FAM channel. *Staphylococcus aureus* as the most important CPS species is additionally identified in channel HEX.

To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to PCR inhibition, an Internal Control (IC) is included in the ROX detection channel.

In case of a negative result due to inhibition of the amplification reaction by the sample matrix, the amplification of the IC is suppressed as well, whereas a true negative result is ensured upon amplification of the IC. This clearly indicates the absence of *Staphylococcus aureus* and coagulase-positive Staphylococci in the sample.

To improve kit stability and handling, the PCR reagents are supplied lyophilised. Uracil-N Glycosylase is already included in the master mix for prevention of DNA-carry-over contamination in the lab.

Properties:

Specificity:
Inclusivity and exclusivity of the **food**proof *Staphylococcus* Detection LyoKit was determined by testing a panel of more than 150 isolates, including 55 isolates of *Staphylococcus aureus* and 23 other coagulase-positive staphylococci: *S. agnetis, S. argenteus, S. cornubiensis, S. delphini, S. hyicus, S. intermedius, S. pseudintermedius, S. schleiferi, S. schweitzeri* and as well as more than 75 non-target strains of coagulase-negative staphylococci (35) and other species (mostly of the closely related genera).

All coagulase-positive staphylococci strains were detected in FAM channel and all *Staphylococcus aureus* strains also in channel HEX/VIC and none of the coagulase-negative staphylococci strains were detected in any channel. The specificity of the **food**proof *Staphylococcus* Detection LyoKit (100%) was confirmed from the inclusivity and exclusivity panel.

Sensitivity:
The LOD 95% of the **food**proof *Staphylococcus* Detection LyoKit was determined for coagulase-positive staphylococci detection in the FAM channel at ≤ 6 GE/reaction and for *Staphylococcus aureus* in the HEX/VIC channel at ≤ 1.5 GE/reaction.

The sensitivity of the *Staphylococcus* detection assay was compared to the ISO 6888-3 culture reference method with various matrices such as infant formula with and without probiotics, whey powder, milk powder, cheeses, ice cream and salad dressings. The matrices were spiked with *Staphylococcus* (*S. aureus* and CPS) at 1-5 CFU/g(ml) and were enriched with Giolitti-Cantoni broth according to ISO 6888-3. Following incubation, extraction was performed utilizing **food**proof StarPrep® Two kit (single-well or 8-Strip), with lysates analyzed using real-time PCR.

A relative detection limit of 1 to 5 cells per 1/10 g sample can be achieved with all relevant kinds of foods. The **food**proof *Staphylococcus* Detection LyoKit detects as few as 10^3 CFU/mL of coagulase-positive staphylococci in the enrichment culture.

PCR LyoKit:

PCR Sensitivity with DNA of *Staphylococcus aureus* (DSM 20232)

Concentration [GE/reaction]	CPS Mean Cq [FAM]	Rate of recovery [FAM]	*S. aureus* Mean Cq [HEX]	Rate of recovery [HEX]	IC Mean Cq [ROX]
50	32.35	100%	32.41	100%	30.56
25	32.96	100%	32.98	100%	30.77
12	33.97	100%	33.89	100%	30.82
6	34.63	100%	34.61	100%	30.94
3	35.71	91%	35.70	100%	30.99
1.5	35.79	91%	35.90	100%	30.42
0.8	36.99	64%	36.59	43%	30.84

PCR Method in Comparison to Culture Reference (ISO 6888-3):

Various commercial products were analyzed. The sample matrices were spiked with different strains of coagulase-positive staphylococci. For each sample matrix, 10 replicates of 1g portions were enriched, and each enrichment culture was analyzed in duplicate. Eight (8) of these replicates were spiked at a fractional positive level (0-1 CFU/g) with *Staphylococcus* and the 2 remaining replicates at a four times higher spiking level. The inoculate count was confirmed by plating on trypticase soy agar for 24 ± 2 hours at 37 ± 1 °C.
The sample enrichment was conducted in Giolitti-Cantoni broth for 24 ± 2 h at 37 ± 1 °C (ISO 6888-3:2003). Afterwards the DNA isolation was carried out with the **food**proof® StarPrep Two Kit using 50 µL of enrichment culture supernatant with Protocol Basic for Liquid Cultures and 25 µL of the extracted DNA were analyzed with the **food**proof *Staphylococcus* Detection LyoKit.

For comparison to standard microbiology procedures, enriched samples were plated on BP (Baird-Parker) and/or RPF (Rabbit Plasma Fibrinogen) agar plates and incubated for 24 ± 2h to 48 ± 2h at 37 ± 1 °C (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).

All PCR results from the tested matrices and strains are comparable to the microbiological method according to ISO 6888-3:2003.

Rate of Recovery after Sample Enrichment

Matrix + 1-5 cfu / sample	CPS [FAM]	*S. aureus* [HEX]	Microbiology (ISO 6888-3)
Skim Milk Powder + *S. aureus* (DSM 799)	90%	90%	90%
Full Cream Milk Powder + *S. aureus* (DSM 20232)	90%	90%	90%
Organic Whey Powder + *S. aureus* (DSM 20232)	70%	70%	70%
Follow-on Milk with Probiotics + *S. aureus* (DSM 20231)	80%	80%	80%
Follow-on Milk with Probiotics + *S. hyicus* (DSM 20459)	60%	0%	60%
Follow-on Milk with Probiotics + *S. schleiferi* (DSM 4808)	70%	0%	70%
Follow-on Milk + *S. intermedius* (DSM 20373)	60%	0%	60%
Cream Cheese + *S. schweitzeri* (DSM 28300)	80%	0%	80%
Chocolate Ice Cream + *S. agnetis* (DSM 23658)	60%	0%	60%
Salad Yogurt Dressing + *S. pseudintermedius* (CCM 7315)	30%	0%	30%

Sensitivity Including Sample Preparation:

foodproof® StarPrep Two Kit DNA Extraction of Infant Formula spiked with *Staphylococcus aureus*

Spiking level [cfu/ml]	CPS Mean Cq [FAM]	*S. aureus* Mean Cq [HEX]	IC Mean Cq [ROX]
10^4	32.98	33.26	31.57
10^3	36.51	37.64	31.78
10^2	-	39.89	32.03
10^1	-	-	31.86
unspiked	-	-	31.75