Validation of the Hygiena's foodproof® *Salmonella* Genus plus Enteritidis & Typhimurium PCR Kit for Raw and Ready-to Cook Meat and Poultry Products and Environmental Samples

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One Health Diagnostics™

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BAX® System X5 | BAX® System Q7 | foodproof® | microproof®

INTRODUCTION:

The serovars *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (STM) are the most frequently reported cause of foodborne illness and account for half of all human *Salmonella* infections. To reduce consumer risk, detection of *Salmonella* spp., including identification of the serovars SE and STM, is critical when managing the food production chain from farm to fork. Several regulations also require testing for these serovars, e.g., EU 1086/2011 (SE and STM in fresh poultry meat) or FDA 21CFR Part 118 (SE in shell eggs). With PCR as the method of choice, products can be released earlier in case of negative results and faster response can be taken in case of contamination, compared to culture methods like ISO 6579-1:2017.

The **food**proof® *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit® has been designed for the robust, reliable and accurate detection and identification of *Salmonella* spp., SE and STM, with one single real-time PCR test. An internal amplification control prevents false negative results due to inhibition. The reaction mix is pre-filled and lyophilized.

PURPOSE:

This study evaluated the **food**proof *Salmonella* Genus plus Enteritidis & Typhimurium Detection LyoKit in combination with **food**proof StarPrep Three Kit for DNA extraction compared to ISO 6579-1:2017 and ISO/TR 6579-3:2014 reference methods according to the requirements of DIN EN ISO 16140-2:2016 and the NordVal International validation protocol.

METHOD:

Sensitivity, level of detection (LOD), and specificity studies were conducted by Campden BRI for the method comparison part of the NordVal International validation study. For sensitivity and LOD studies, artificially contaminated samples of raw meat and poultry, ready-to-cook meat and poultry products, and environmental samples were incubated for 16-20 hours at 37 °C, following sample preparation according to ISO 6887 methods. After incubation, DNA extraction was performed with the **food**proof StarPrep® Three Kit in both single tube and 8-strip formats followed by real-time PCR analysis. Specificity testing included 25 *S.* Enteritidis, 25 *S.* Typhimurium, 75 *Salmonella* spp. strains, and 30 non-target strains.

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SENSITIVITY STUDY:

The sensitivity study assessed the ability of the method to detect the analyte in different matrices within the chosen categories.

Three categories were included in this study: raw and ready-to-cook meat products, raw and ready-to-cook poultry products and environmental samples. A minimum of 60 samples for each category and each target (*S.* Enteritidis, *S.* Typhimurium and *Salmonella* spp.) were analyzed by both the alternative and reference method (ISO 6579-1:2017 and ISO TR 6579-3:2014). The samples inoculated with *S.* Enteritidis and *S.* Typhimurium were combined to give the data set for *Salmonella* spp. as the assay enables the detection of the three targets in one single PCR reaction. To ensure that samples were contaminated with the two specific target *Salmonella* serotypes (Enteritidis and Typhimurium), artificial contamination of samples was carried out using a seeding protocol. All samples were inoculated at a level of 1-5 CFU/test portion, with a maximum of 7 CFU/test portion.

The observed values for the deviating results (ND-PD) and (ND+PD) are below the acceptability limits and were thus met for each individual category and for all the combined categories for all three targets in the **food**proof *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit - *S.* Enteritidis, *S.* Typhimurium and *Salmonella* spp.

Table 1a. *S.* Enteritidis - Overview of the calculated sensitivity parameters per category & type

Category	Type	PA	NA	PD	ND	FP	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
Raw meat & ready-to-cook meat products	Fresh meats (unprocessed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Raw poultry & ready-to-cook poultry products	Fresh meats (unprocessed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Environmental samples	Dust & residues	10	10	0	0	0	100	100	100	0.0
	Cleaning & process waters	10	10	0	0	0	100	100	100	0.0
	Surface samples	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
	OVERALL	**90**	**90**	**0**	**0**	**0**	**100**	**100**	**100**	**0.0**

Table 1b. *S.* Typhimurium - Overview of the calculated sensitivity parameters per category & type

Category	Type	PA	NA	PD	ND	FP	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
Raw meat & ready-to-cook meat products	Fresh meats (unprocessed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Raw poultry & ready-to-cook poultry products	Fresh meats (unprocessed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	10	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
Environmental samples	Dust & residues	10	10	0	0	0	100	100	100	0.0
	Cleaning & process waters	10	10	0	0	0	100	100	100	0.0
	Surface samples	10	10	0	0	0	100	100	100	0.0
	Total	30	30	0	0	0	100	100	100	0.0
	OVERALL	**90**	**90**	**0**	**0**	**0**	**100**	**100**	**100**	**0.0**

Table 1c. *Salmonella* spp. - Overview of the calculated sensitivity parameters per category & type

Category	Type	PA	NA	PD	ND	FP	SE alt (%)	SE ref (%)	RT (%)	FPR (%)
Raw meat & ready-to-cook meat products	Fresh meats (unprocessed)	20	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	20	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	20	10	0	0	0	100	100	100	0.0
	Total	60	30	0	0	0	100	100	100	0.0
Raw poultry & ready-to-cook poultry products	Fresh meats (unprocessed)	20	10	0	0	0	100	100	100	0.0
	Ready-to-cook (processed)	20	10	0	0	0	100	100	100	0.0
	Ready-to-eat & ready-to-reheat products	20	10	0	0	0	100	100	100	0.0
	Total	60	30	0	0	0	100	100	100	0.0
Environmental samples	Dust & residues	20	10	0	0	0	100	100	100	0.0
	Cleaning & process waters	20	10	0	0	0	100	100	100	0.0
	Surface samples	20	9	0	0	1	100	100	100	11.1
	Total	60	29	0	0	1	100	100	100	3.4
	OVERALL	**180**	**89**	**0**	**0**	**1**	**100**	**100**	**99.4**	**1.1**

PA: number of positive results obtained with both the alternative and the reference method; **NA:** number of negative results obtained with both the alternative and the reference method; **ND:** number of obtained results that are negative with the alternative method and positive with the reference method; **PD:** number of obtained results that are positive with the alternative method and negative with the reference method; **FP:** number of false positives; **RT:** relative trueness; **SE alt:** sensitivity for the alternative method; **SE ref:** sensitivity for the reference method; **FPR:** false positive rate.

LEVEL OF DETECTION & RELATIVE LEVEL OF DETECTION STUDY:

The level of detection (LOD_{50}) is the smallest number of culturable microorganisms that can be detected 50% of the time in a given sample. The relative level of detection (RLOD) is the ratio of the LOD of the alternative method and the LOD of the reference method.

For each of the three categories, a single sample type was tested with three different levels of contamination. One set of samples included 5 uninoculated samples (0 CFU/test portion), 20 low level inoculated samples (0.2-2 CFU/test portion) to obtain fractional positive results, and 5 high level inoculated samples (2-5 CFU/test portion). For inoculation, a different strain of *Salmonella* was used for each sample type.

The LOD_{50} varied from 0.6 – 1.0 CFU/test portion. The RLOD values (using the confirmed alternative method results) for all three targets of the **food**proof *Salmonella* Genus plus Enteritidis and Typhimurium Detection LyoKit meet the acceptability limit, which is 1.5 for paired studies.

Table 2a. *S.* Enteritidis - LOD_{50} and RLOD after confirmation of the alternative method results

Category	LOD_{50} CFU per portion	Lower confidence limit CFU per portion	Upper confidence limit CFU per portion	RLOD using the confirmed alternative method results
Raw poultry and ready-to-cook poultry products	0.792	0.451	1.390	1.000
Environmental samples	1.040	0.584	1.852	1.000
Combined	0.909	0.608	1.359	1.000

Table 2b. *S.* Typhimurium - LOD_{50} and RLOD after confirmation of the alternative method results

Category	LOD_{50} CFU per portion	Lower confidence limit CFU per portion	Upper confidence limit CFU per portion	RLOD using the confirmed alternative method results
Raw meat and ready-to-cook meat products	0.702	0.402	1.225	1.000

Table 2c. *Salmonella* spp. - LOD_{50} and RLOD after confirmation of the alternative method results

Category	LOD_{50} CFU per portion	Lower confidence limit CFU per portion	Upper confidence limit CFU per portion	RLOD using the confirmed alternative method results
Raw meat and ready-to-cook meat products	0.728	0.419	1.267	1.000
Raw poultry and ready-to-cook poultry products	0.792	0.451	1.390	1.000
Environmental samples	0.570	0.299	1.089	1.000
Combined	0.706	0.503	0.990	1.000

INCLUSIVITY & EXCLUSIVITY STUDY:

A total of 25 *S.* Enteritidis, 25 *S.* Typhimurium and 75 *Salmonella* spp., and 30 non-target strains were included for specificity testing. Strains used for inclusivity tests were grown overnight in nutrient broth and inoculated into BPW at a level 100 times greater than the minimum limit of detection. After inoculation, the inclusivity samples were incubated for 16 h at 37 ± 1 °C prior to analysis using the alternative method and conducting the confirmatory tests. All 30 non-target strains were grown overnight in an appropriate broth and inoculated into BPW to achieve a concentration of 10^5 CFU/mL. Following inoculation, the exclusivity samples were incubated for 16 h at 37 ± 1 °C before testing using the alternative method. Specificity claims for the assay and extraction method combination successfully detected and identified 100% of target strains in the respective detection channels with 100% exclusivity of non-target strains.

SIGNIFICANCE:

This validation data demonstrates that the evaluated multiplex real-time PCR assay is a rapid and reliable alternative method for the detection of *Salmonella* spp., *Salmonella* Enteritidis and *Salmonella* Typhimurium in raw and ready-to-cook meat and poultry products and environmental samples. Based on these results and an interlaboratory study the assay received a NordVal validation certificate (NordVal No. 055).