

Validation of a New TaqMan® Real-Time PCR Method for the Detection of Salmonella enterica in a Variety of Food Samples Using a Single 18-Hour Enrichment Protocol

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INTRODUCTION

The TaqMan® Salmonella enterica test from Applied Biosystems is a qualitative assay for the detection of Salmonella species in foods and feeds. It utilises the highly sensitive and specific combination of TaqMan chemistry and real-time PCR for the amplification and detection of Salmonella DNA.

AIM

The aim of the validation is to demonstrate that the TaqMan Salmonella enterica method is a rapid alternative to the reference culture method without compromising on sensitivity or specificity.

METHOD

The TaqMan Salmonella method was validated as an alternative method for the detection of Salmonella species in foodstuffs and feedstuffs following the ISO 16140:2003 validation requirements.

The TaqMan Salmonella method was performed in accordance with the manufacturers instructions. Samples were enriched in a single-step using Buffered Peptone Water (BPW) at 37°C for 18 hours. This was followed by DNA extraction of 1ml of the BPW and real-time PCR to amplify and detect the target from the Salmonella enterica genome. Total time to result with the Applied Biosystems test was less than 21 hours. All samples were tested in parallel following the ISO 6579 method as a reference. Total time to result with the ISO reference method was approximately 68 hours.

The study compared the protocols for the detection of Salmonella species in a variety of naturally and artificially contaminated foods within 5 categories; meat products, dairy products, seafood and vegetable products, egg products and feedstuffs. Of the 333 samples analysed 155 were positive and 178 were negative for Salmonella species. A more detailed breakdown of these matrices is shown in table 1.

As part of the validation study inclusivity and exclusivity across a number of strains was examined. Artificially contaminated samples were spiked using strains which had been stressed using different treatments.

The validation involved an inter-laboratory study which included 10 European laboratories to demonstrate the reproducibility and robustness of the assay. Results were analysed according to the principles of EN ISO 16140 and statistical analysis performed.

RESULTS

The validation demonstrated across a range of foods that the TaqMan Salmonella enterica protocol is comparable to the ISO reference method. The relative accuracy of the alternative method was calculated to be 98.5%, the relative specificity 99.4% and the relative sensitivity 97.4%.

All 58 Salmonella target strains gave positive results, demonstrating that the alternative method is capable of detecting target analytes from a broad range of strains. All 36 non-Salmonella strains successfully gave negative results with the alternative method, demonstrating that there is no interference from the non-target strains. This inclusivity and exclusivity testing highlights the level of specificity and accuracy that the TaqMan Salmonella enterica kit offers.

The relative detection level, that is the smallest number of culturable micro-organisms that can be detected in the sample in 50% of occasions, has been shown to be similar for the alternative and standard methods. Table 2 illustrates relative detection levels according to the Spearman-Kärber test.

Results from the inter-laboratory study showed comparable results illustrating that the method is reproducible amongst different operators and laboratories irrespective of previous experience.

The TaqMan Salmonella enterica practicability was also evaluated according to the AFNOR criteria. It was shown that less than one day of training is required for technicians with no prior experience of the method.

Table 1. Breakdown Per Category and Type

Category	Type	Positives (number)	Negatives (number)	Total (number)
Meat Products	Poultry	17	11	28
	Raw meat (pork, beef)	8	14	22
	Delicatessen	5	18	23
	Total	30	43	73
Dairy Products	Raw milk	9	16	25
	Raw milk cheese	11	11	22
	Milk powder, ice cream, others	11	8	19
	Total	31	35	66
Seafood & Vegetables	Frozen vegetables	11	10	21
	Raw fish	12	8	20
	Various products	10	12	22
	Total	33	30	63
Egg Products	Raw egg	15	10	25
	Mayonnaise	7	8	15
	Egg powder, egg-based products	9	16	25
	Total	31	34	65
Feedstuffs	Pet food	11	12	23
	Raw meat	10	12	22
	Dehydrated products, meat flours	9	12	21
	Total	30	36	66
TOTAL		155	178	333

Table 2. Relative Detection Level

Strain / Matrix Pairs	Relative Detection Level cfu/25g	
	Reference Method	Alternative Method
Ground Beef / <i>Salmonella infantis</i> 14	0.434 [0.284; 0.662]	0.496 [0.310; 0.794]
Raw Milk / <i>Salmonella typhimurium</i> 305	0.447 [0.120; 1.666]	0.447 [0.120; 1.666]
Raw Fish / <i>Salmonella saintpaul</i> F31	0.525 [0.178; 1.550]	0.698 [0.379; 1.286]
Egg Product / <i>Salmonella enteritidis</i> 2532	0.545 [0.185; 1.608]	0.545 [0.185; 1.608]
Dog Food / <i>Salmonella agona</i> A00V038	0.346 [0.112; 1.068]	0.308 [0.100; 0.953]

CONCLUSION

The TaqMan Salmonella enterica method from Applied Biosystems is an easy-to-use rapid alternative to the conventional ISO 6579 culture method. The AFNOR validation study found the alternative method to be specific with a similar limit of detection to the culture method. There were no substantial differences between the TaqMan Salmonella method and the reference method for the detection of Salmonella species. The method was granted AFNOR validation against EN ISO 16140 in May 2007.

TRADEMARKS/LICENSING

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