APPLICATION NOTE

Use of an Antibiotic-free Enrichment Medium For Detection of *Salmonella* in Cheese Samples Using the Thermo Scientific SureTect Salmonella Species PCR Assay

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Introduction

NF VALIDATION™ certification by AFNOR certification has been granted for the Thermo Scientific™ SureTect™ Salmonella species PCR Kit for the detection and differentiation of *Salmonella* species from food, feed and environmental samples. The PCR assay workflow is conducted using either the Applied Biosystems™ 7500 Fast Food Safety Real-Time PCR System (Applied Biosystems™ 7500 Fast Real-Time PCR Instrument and Applied Biosystems™ RapidFinder Express Software v2.0 of higher) or the Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR System (Applied Biosystems™ QuantStudio 5 Food Safety Real-Time PCR Instrument and Thermo Scientific™ RapidFinder™ Analysis Software v1.0 or higher), shown in Figure 2.

The validated method for dairy products requires enrichment of these matrices with novobiocin-containing media. The addition of antibiotics to enrichment media allows for the reliable recovery of *Salmonella* from products that contain high levels of background flora. Some dairy products, including those that are pasteurized, do not require the addition of antibiotics to the enrichment media as growth of *Salmonella* is not negatively impacted by high levels of competing microorganisms.

Figure 1. Variety of cheeses

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The following data demonstrates the performance of the SureTect Salmonella species assay (alternative method) versus the ISO reference method when detecting *Salmonella* from cheese samples using an enrichment medium without novobiocin.

Materials and methods

Two studies were conducted, comparing the alternative method to the reference method using soft cheese (Mozzarella), semi-hard cheese and blue cheese matrices.

In the first study, 18 cheese samples were tested with the alternative method using prewarmed Buffered Peptone Water (BPW) for enrichment at 37°C while the second study tested another 18 samples using prewarmed-BPW for enrichment at 41.5°C (Figure 3). Both studies were compared to the ISO reference method (Figure 4) with an unpaired study design.

Four samples were artificially contaminated with *Salmonella* spp. and two samples were left unspiked for each of the matrices tested, per method. The contamination level for all isolates was 2-3 CFU per 25 g sample.

All alternative method samples were confirmed with the culture confirmation method using a direct streak of

Figure 2. Thermo Scientific SureTect System with Applied Biosystems™ SimpliAmp Thermal Cycler and Thermo Scientific SureTect Salmonella species PCR Assay

enrichment broth onto *Brilliance*™ Salmonella Agar (Thermo Fisher Scientific) or using a selective enrichment in RVS before inoculating onto *Brilliance* Salmonella Agar for high background matrices (Figure 3).

The alternative method was run using the Applied Biosystems 7500 Fast Food Safety Real-Time PCR System.

Figure 3. Process flow for the alternative methods and confirmation method

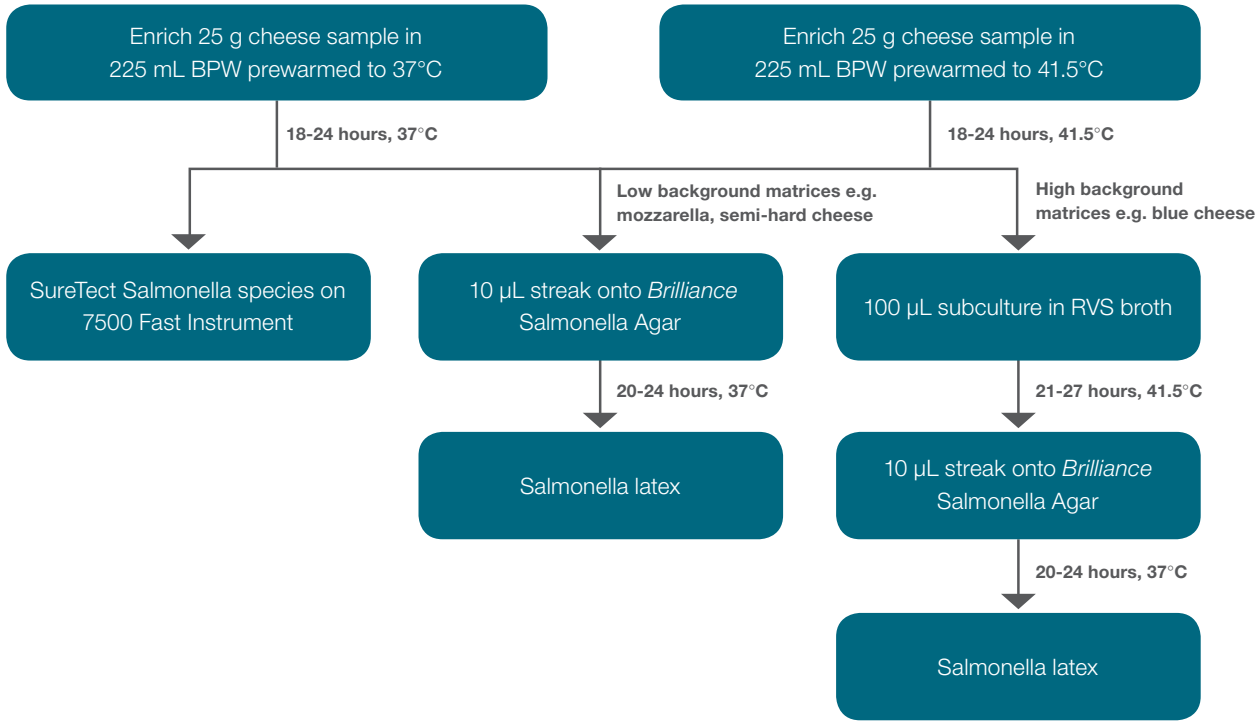
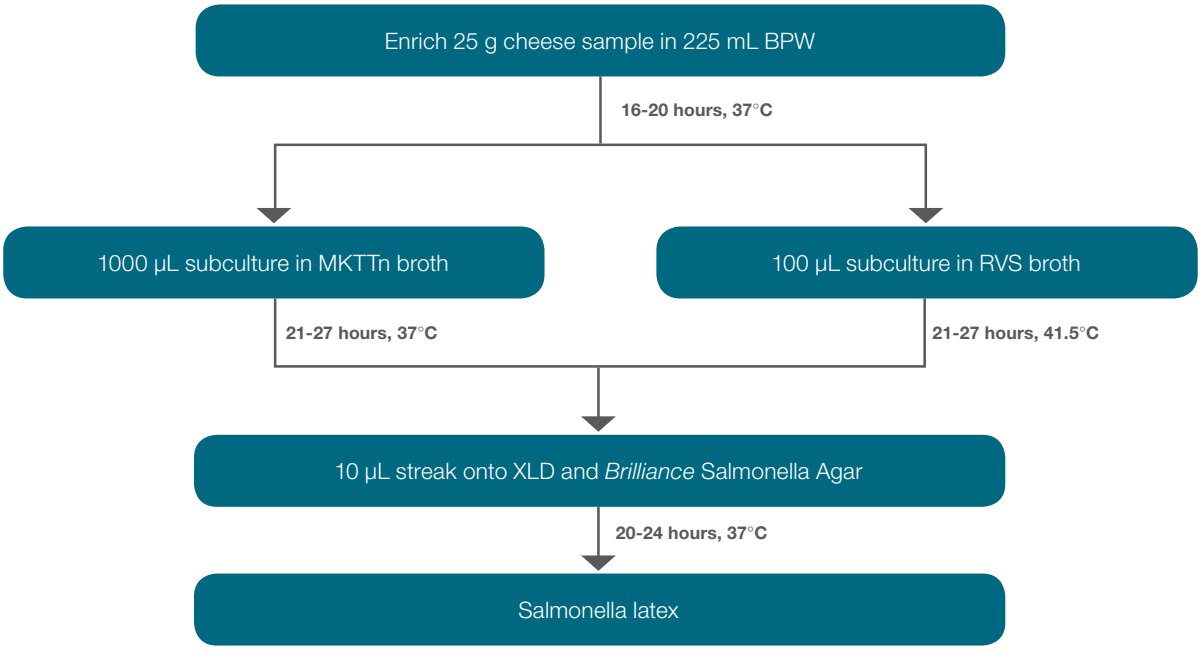


Figure 4. Process flow for the reference method



Results

Table 1. Method agreement results for the alternative and the reference methods

Agreement	Alternative method	
	37°C	41.5°C
PA	12	12
NA	6	6
PD	0	0
ND	0	0

Key:

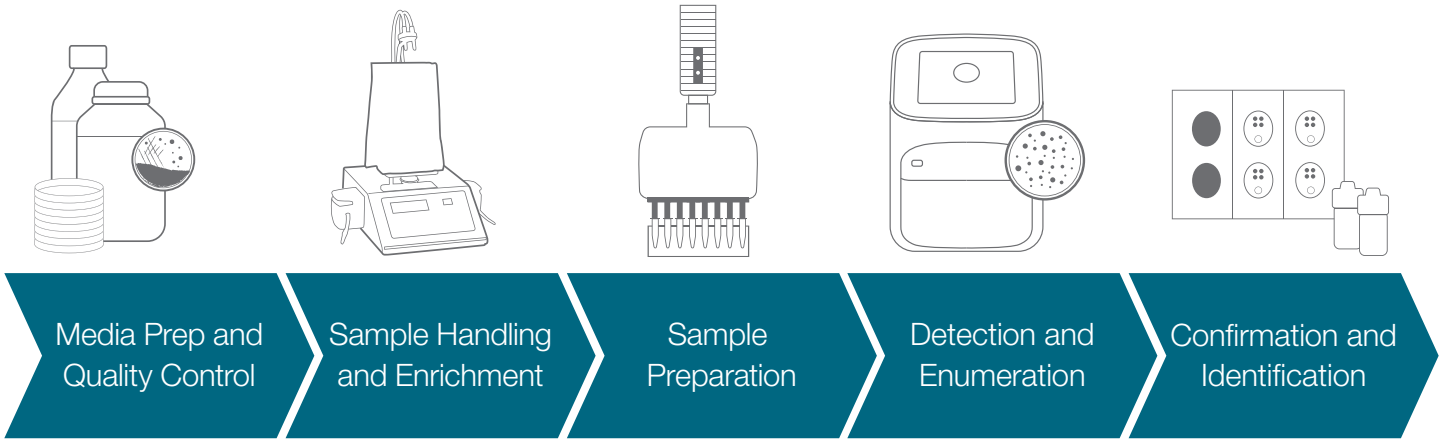
Agreement	Alternative method PCR Result	Alternative method confirmation result	ISO reference method result
PA	+	+	+
NA	-	-	-
PD	+	+	-
ND	-	-	+

PA: Positive Agreement NA: Negative Agreement PD: Positive Deviation ND: Negative Deviation

The alternative methods had 100% agreement with the reference method for 18 hours enrichment and 24 hours enrichment (Table 1). No deviations were observed, indicating that the alternative method performed equivalently to the reference method, even for the high background matrices used in this study. It is noted that some matrices with particularly high background flora, such as raw milk cheese, may still require the addition of novobiocin to support recovery of *Salmonella* and that performance should be evaluated with BPW without novobiocin on a case-by-case basis.

Conclusion

The results show that the alternative method, using prewarmed broth without antibiotics for enrichment, is a suitable and comparable alternative to the reference method when detecting *Salmonella* from cheese samples that do not contain high levels of background flora.



References

1. ISO 6579-1:2017 Microbiology of the food chain-Horizontal method for the detection, enumeration and serotyping of *Salmonella*-Part 1: Detection of *Salmonella* spp.

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