APPLICATION NOTE

Harmonized Sample Preparation and Enrichment for the SureTect Cronobacter Species and Salmonella Species PCR Assays with Powered Infant Formula

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Figure 1. Applied Biosystems 7500 Fast Food Safety Real-Time PCR System and Thermo Scientific Salmonella species and Cronobacter species PCR Kits.

Introduction

The Thermo Scientific™ SureTect™ Cronobacter species and Thermo Scientific™ SureTect™ Salmonella species PCR Assays are both validated under NF VALIDATION™ certification by AFNOR for the detection of *Cronobacter* and *Salmonella* from powered infant formula (PIF) samples. The *Cronobacter* assay is validated for use with 10 g samples using BPW and for 300 g using BPW with 6 mg/L vancomycin as the primary enrichment broths. Addition of antibiotics for large sample sizes is necessary to prevent lowering of pH during enrichment by probiotic organisms which causes die-off of *Cronobacter.*

The *Salmonella* assay is validated for use with all foods at a sample size of 25 g using BPW without antibiotics.

Both assays are validated for use with the Applied Biosystems™ 7500 Fast Food Safety System (see Figure 1) with RapidFinder™ Express Software v2.0 and Applied Biosystems™ QuantStudio™ 5 Food Safety System with Thermo Scientific™ RapidFinder Analysis Software v1.0.

To enable testing of both *Cronobacter* and *Salmonella* from the same enrichment of large 300 g sample sizes, a study was conducted to confirm performance of the SureTect Salmonella Assay with BPW + 6 mg/L vancomycin.

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Materials and methods

Sample preparation

Six different powered infant formula products, including those containing probiotic organisms, were weighed into test portions and processed according to the protocols described in Figures 2 and 3.

Samples were spiked with isolates that had been subjected to injury by desiccation. The injury and spike levels were calculated by serially diluting the desiccated culture in MRD then enumerating on both a non-selective and a selective agar (Tables 1 and 2). The injury calculation was conducted as follows as per AFNOR NF VALIDATION guidelines:

Injury = $\log_{10}$ non-selective agar count – $\log_{10}$ selective agar count

Lysis protocol

The SureTect lysis protocol is harmonized for both *Cronobacter* and *Salmonella* SureTect PCR assays. To a pre-filled tube of Lysis Reagent 1, 10 µL of Proteinase K was added followed by 10 µL of enriched sample. The Lysis Tubes were capped and heated to 37°C for 10 minutes then 95°C for 5 minutes. Samples were allowed to cool at room temperature for 2 minutes to allow for safe handling before loading into the PCR instrument.

Culture confirmation

All SureTect method samples were tested using the appropriate chromogenic media[1,2] to confirm PCR positive and negative results in accordance with Figure 2.

Table 1. *Cronobacter* isolate injury and spike levels.

Organism name	Injury	CFU/sample
Cronobacter muytjensii	1.35	4.37
Cronobacter sakazakii	0.64	5.00
Cronobacter sakazakii	0.67	3.36
Cronobacter dublinensis subsp. *dublinensis*	1.15	7.81
Cronobacter sakazakii	1.05	12.25
Cronobacter sakazakii	1.15	19.86
Cronobacter turicensis	1.19	8.69
Cronobacter sakazakii	0.85	8.85
Cronobacter sakazakii	0.52	8.22
Cronobacter malonaticus	-	9.00
Cronobacter sakazakii	1.18	8.52
Cronobacter sakazakii	0.73	9.60
Cronobacter dublinensis subsp. *lausannensis*	1.33	7.32
Cronobacter sakazakii	1.33	5.76
Cronobacter sakazakii	0.68	6.93

Table 2. *Salmonella* isolate injury and spike levels.

Organism name	Injury	CFU/sample
Salmonella Newport	0.86	3.18
Salmonella Montevideo	0.78	3.06
Salmonella Binza	0.96	3.84
Salmonella Napoli	0.82	3.25
Salmonella Ohio	1.07	2.64
Salmonella Derby	1.09	2.94
Salmonella Livingstone	0.79	2.65
Salmonella Agona	0.90	1.82
Salmonella Anatum	1.36	2.11
Salmonella Indiana	0.54	3.65
Salmonella Mbandaka	0.68	2.28
Salmonella Typhimurium	0.76	3.21

Figure 2. SureTect method and confirmation method workflows.

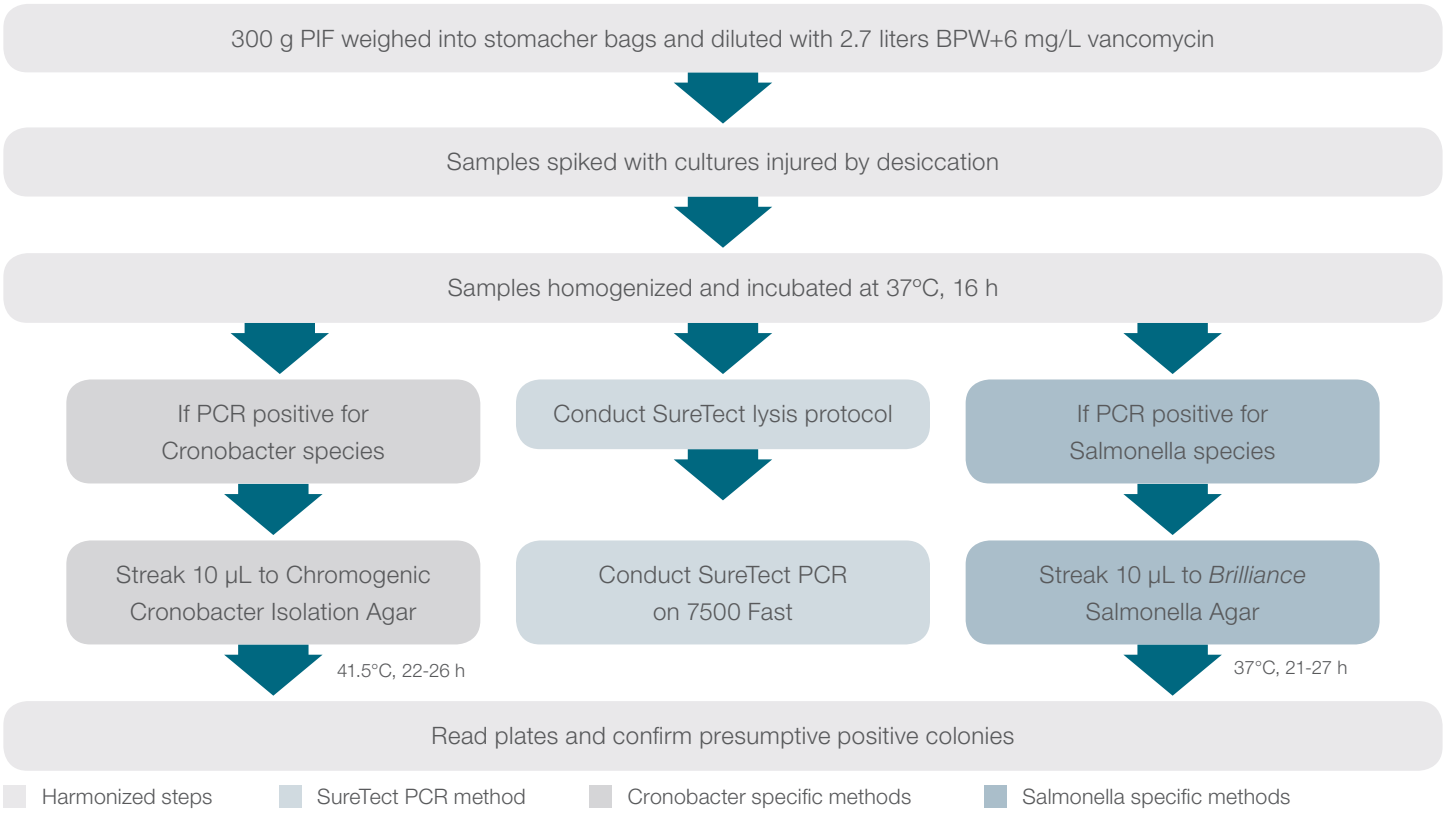
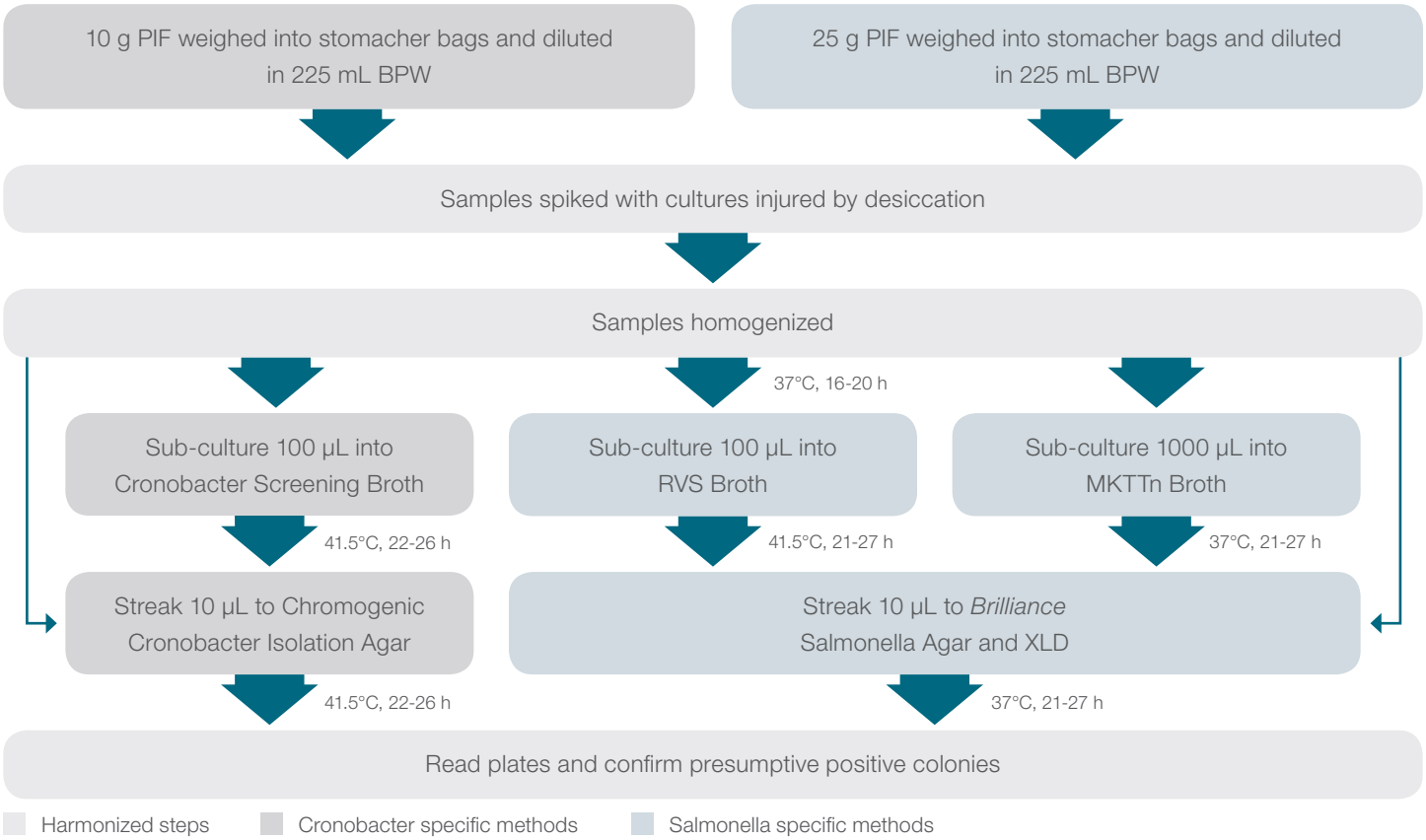


Figure 3. ISO reference method workflows.



Results

Table 3. Method agreement between the candidate presumptive and culture confirmation result.

SureTect Assay Method	Method Agreement				
	PA	NA	PD	ND	ND-PD
Cronobacter	12	5	3	0	-3
Salmonella	11	4	1	0	-1

PA-Positive agreement (candidate +, reference +)
NA-Negative agreement (candidate -, reference -)
PD-Positive deviation (candidate +, reference -)
ND-Negative deviation (candidate -, reference +)

Both assays achieved zero negative deviations from the unpaired ISO reference method samples (Table 3). The total number of positive deviations for the PCR assays was three for the SureTect *Cronobacter* Assay and one for the SureTect *Salmonella* Assay. This indicates that the SureTect method for 300 g samples performed better than the ISO reference method for 25 g samples.

No false positive or false negative results were achieved for the 300 g samples when culture confirmation methods were conducted.

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Conclusions

Using the harmonized sample preparation and enrichment protocol for 300 g sample sizes, both the SureTect Cronobacter and Salmonella Assays succeeded in achieving 100% sensitivity according to the culture confirmation methods applied to these samples.

When compared to the ISO reference methods which tested a smaller sample size of 25 g, the SureTect methods achieved superior performance, confirming that both the harmonized sample preparation and enrichment protocol for the SureTect Cronobacter and Salmonella assays using BPW + 6 mg/L vancomycin is suitable for use with PIF containing probiotic organisms for samples of up to 300 g.

References

1. ISO 22964:2017 Microbiology of the food chain—Horizontal method for the detection of *Cronobacter* spp.
2. 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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993-315
LT2422A
November 2018

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