Food Safety

Harmonized PCR Detection of *Salmonella* and *Cronobacter* species

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Introduction

Annually, *Salmonella* and *Cronobacter* spp. cause 200 million infections worldwide[1]. One such source of infection is powdered infant formula (PIF), which is especially concerning for neonates, with case mortality reported to be 50-80%[2]. Sensitive, easy, and reliable methods to detect the presence of *Salmonella* and *Cronobacter* spp. in PIF are of fundamental importance to reduce mortality rates.

The Thermo Scientific™ SureTect™ Salmonella species PCR Assay and Thermo Scientific™ SureTect™ Cronobacter species PCR Assay were evaluated according to ISO 16140-2[3] and AOAC Appendix J[4] guidelines for the detection of *Salmonella* and *Cronobacter* species from 375 g PIF samples from the same enrichment (Figures 1 and 2) against the ISO 22964:2017[5] and ISO 6579-1:2017[6] reference methods. The study design is outlined below.

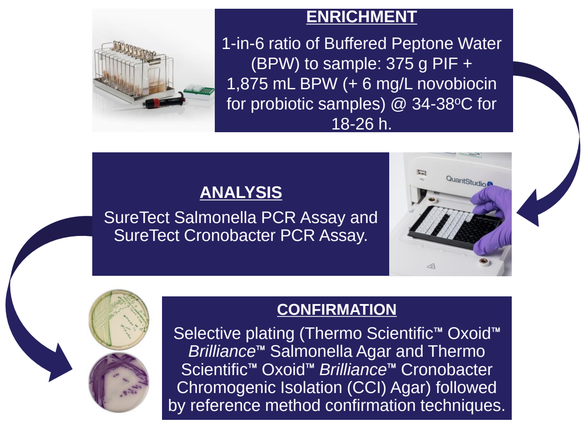
Study design

SENSITIVITY
- A total of 65 samples were analyzed for *Salmonella* spp. in an unpaired study.
- A total of 66 samples were analyzed for *Cronobacter* spp. in an unpaired study.

RELATIVE LEVEL/PROBABILITY OF DETECTION (RLOD/POD)
- Thirty PIF samples were analyzed following an unpaired study design for both assays against the ISO 6579 and ISO 22964 reference methods.

Method workflow

Figure 1: *Salmonella* and *Cronobacter* harmonized SureTect workflow



Key method benefits

Figure 2: Key benefits of the harmonized SureTect Salmonella and Cronobacter PCR Assay workflows

One Sample
- Large sample size: **Up to 375 g**
- Low dilution ratio: **1-in-6**

Ease of Enrichment
- Antibiotic addition **only** for probiotic samples

Certified
- **ISO 16140-2**
- **AOAC PTM**

Results

The required acceptability limits/criteria were met successfully for all studies for both SureTect PCR Assay methods as outlined in Tables 1 and 2. For the RLOD/POD study all differences in POD were in favor of the alternative method with the SureTect Salmonella PCR Assay showing superior performance to ISO 6579 during the POD study for 375 g PIF with probiotics as shown by the positive POD confidence interval in Table 2.

Table 1: Sensitivity study results for SureTect Salmonella vs. ISO 6579 (A) and SureTect Cronobacter vs. ISO 22964 (B): PA = Positive Agreement, NA = Negative Agreement, PD = Positive Deviation, ND = Negative Deviation

A	ISO 6579 Positive	ISO 6579 Negative	Sensitivity SureTect Salmonella	Sensitivity ISO 6579
SureTect Salmonella Positive	27 (PA)	7 (PD)	97.1%	80.0%
SureTect Salmonella Negative	1 (ND)	30 (NA)		

B	ISO 22964 Positive	ISO 22964 Negative	Sensitivity SureTect Cronobacter	Sensitivity ISO 22964
SureTect Cronobacter Positive	19 (PA)	7 (PD)	86.7%	76.7%
SureTect Cronobacter Negative	4 (ND)	36 (NA)		

Table 2: RLOD/POD study results for SureTect Salmonella and Cronobacter vs. ISO 6579 & 22964, respectively

Matrix	Assay	Timepoint	Difference in POD	95% POD Confidence Interval	RLOD	Acceptance
 375 g PIF with probiotics	SureTect Salmonella	18 h	0.00	-0.43, 0.43	0.843	PASS
			0.05	-0.23, 0.32		
			0.00	-0.43, 0.43		
	SureTect Cronobacter		0.00	-0.43, 0.43	0.868	
			0.35	0.07, 0.57		
			0.40	-0.12, 0.77		
 375 g PIF without probiotics	SureTect Salmonella		0.00	-0.43, 0.43	1.000	
			0.00	-0.28, 0.28		
			0.00	-0.43, 0.43		
	SureTect Cronobacter		0.00	-0.43, 0.43	1.000	
			0.00	-0.28, 0.28		
			0.00	-0.43, 0.43		

Conclusions

Simple — *One enrichment, two results*
- One sample with non-proprietary enrichment media and low dilution ratio to maximize laboratory throughput and efficiency.

Accurate — *Equivalent to ISO reference methods*
- Performance data shows equivalent or superior performance to the gold standard ISO reference methods.

Proven — *NF VALIDATION & AOAC PTM certified*
- Granted certification by AOAC PTM and NF VALIDATION according to ISO 16140-2.

References

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3. ISO 16140-2:2016: Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (2016), ISO, https://www.iso.org/standard/54870.html (accessed May 2024)
4. Official Methods of Analysis of AOAC INTERNATIONAL (2019) 21st Ed., Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, AOAC INTERNATIONAL, Gaithersburg, MD, http://www.eoma.aoac.org/app_j.pdf (accessed May 2024)
5. 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., https://www.iso.org/standard/56712.html (accessed May 2024)
6. ISO 22964:2017: Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp., ISO, https://www.iso.org/standard/64708.html (accessed May 2024)

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