Detection of Multiple Pathogens from Powdered Infant Formula using the SureTect PCR Assay Range

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INTRODUCTION

To safeguard young infants, reliable detection methods using PCR provide valuable tools to ensure powdered infant formula (PIF) is free from pathogenic bacteria.

PURPOSE

Studies were performed to evaluate the performance of three Thermo Scientific™ SureTect™ PCR Assay workflows for detection of *Salmonella* spp., *Cronobacter* spp., and *Staphylococcus aureus* from PIF.

METHOD

The probability of detection (POD) according to the AOAC Guidelines and the relative limit of detection (RLOD) according to the ISO 16140-2:2016 were generated with 25 g and 375 g test portions for *Salmonella* spp., and 10 g and 300 g test portions for *Cronobacter* spp.

A total of 238 samples was tested in unpaired studies during the ISO 16140-2:2016 sensitivity studies of SureTect PCR workflows (NF VALIDATION). This comprised 97 unpaired PIF samples for *Salmonella* (25 g and 375 g test portions) and 141 for *Cronobacter* (10 g and 300 g test portions).

A paired sensitivity study testing fifty-four 25 g test portions of PIF was conducted for the SureTect Staphylococcus aureus PCR Assay and performance compared to the ISO reference method.

Figure 1. Infant Formula and the QuantStudio 5 Food Safety System

The sample sizes of infant formula recommended for testing with the SureTect range varies between 10 g and 375 g; sample enrichment conditions may differ per sample size and per pathogen to be tested for. All three SureTect assays can be run with the Applied Biosystems™ 7500 Fast and Applied Biosystems™ QuantStudio™ 5 Food Safety Systems

RESULTS

Table 1. POD data for the *Salmonella* and *Cronobacter* PCR assays using data from the ISO RLOD studies

Assay	Test portion	Inoculum	dPOD (LCL, UCL)	RLOD
Salmonella	25 g	Low (N=20)	0.20 (-0.10, 0.45)	1.000
		High (N=5)	0.00 (-0.43, 0.43)	
	375 g	Low (N=20)	0.50 (0.21, 0.70)	1.195
		High (N=5)	0.60 (-0.14, 0.77)	
Cronobacter	10 g	Low (N=20)	0.00 (-0.28, 0.28)	0.148
		High (N=5)	0.00 (-0.43, 0.43)	
	300 g	Low (N=20)	-0.05 (-0.32, 0.23)	0.195
		High (N=5)	-0.40 (-0.43, 0.19)	1.482

dPOD: Probability of detection between the reference method and confirmed candidate method, LCL: Lower control limit, UCL: Upper control limit

Table 2. Unpaired study data according to ISO validation studies for *Salmonella* and *Cronobacter* and paired study data for *S. aureus*

Organism	Test portion	PA	NA	PD	ND	FN	FP
Salmonella	25 g	33	29	0	1	0	1
	375 g	17	11	2	3	0	0
Cronobacter	10 g	29	32	1	0	0	2
	300 g	23	38	9	3	0	4
S. aureus	25 g	35	16	0	0	1	2

All of the data displayed above was generated using the QuantStudio 5 Food Safety System.
Test matrices included products with and without probiotics
PA: Positive Agreement NA: Negative Agreement PD: Positive Deviation
ND: Negative Deviation FN: False Negative FP: False Positive

The POD calculation indicated that the *Salmonella* PCR workflow was more reliable than the reference method as the dPOD confidence intervals did not contain zero and in the case of 375 g was skewed in favour of the candidate method (Table 1). The *Salmonella* sensitivity study showed comparable performance to ISO 6579; two positive and four negative deviations were observed (Table 2).

The POD values of the *Cronobacter* study confirmed that the candidate method performed comparably to the ISO reference method (Table 1). The SureTect Cronobacter PCR Assay showed improved performance to the ISO 22964 reference method (Table 2); ten positive and three negative deviations were observed in the sensitivity studies.

The *S. aureus* paired study showed that the candidate presumptive result was comparable to the traditional plating method.

CONCLUSIONS

The SureTect PCR workflows provide rapid and reliable methods for the detection of multiple pathogens from powdered infant formula samples ranging from 10 g to 375 g.

The AOAC POD and ISO 16140-2 RLOD study designs can be calculated using the same data sets and provide complementary information of the performances of the detection method. With both calculations, SureTect PCR Assays are considered equivalent to the reference method.

TRADEMARKS/LICENSING

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