thermoscientific

STUDY REPORT

Thermo Scientific SureTect Cronobacter species PCR Assay Workflow NF VALIDATION ISO 16140-2:2016

Jessica Williams, Thermo Fisher Scientific,
Wade Road, Basingstoke, Hampshire, RG24 8PW, UK

Summary

The Thermo Scientific™ SureTect™ Cronobacter species PCR Assay (PT1060A) (alternative method) has been certified by NF VALIDATION (certification reference UNI 03/11-12/15) for the detection of *Cronobacter* spp. from powdered infant formula (PIF) (10 g and 300 g) and production environment samples. The following report gives a summary of the studies performed as part of the NF VALIDATION.

Methodology

Study	Reference method
Initial validation PCR analysis was conducted using the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument	**ISO/TS 22964:2006** Milk and milk products—Detection of *Enterobacter sakazakii* and **ISO/DIS 22964:2015** Milk and milk products—Detection of *Enterobacter sakazakii* simultaneously
Extension study To incorporate the Applied Biosystems™ 7500 Fast Food Safety System (7500 Fast Real-Time PCR Instrument and Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or higher))	**ISO/TS 22964:2006** Milk and milk products—Detection of *Enterobacter sakazakii* and **ISO/DIS 22964:2015** Milk and milk products—Detection of *Enterobacter sakazakii* simultaneously
Extension study To incorporate the Applied Biosystems™ QuantStudio™ 5 Food Safety System (Applied Biosystems QuantStudio 5 Real-Time PCR Instrument and RapidFinder™ Analysis Software version 1.0)	**ISO 22964:2017:** Microbiology of the food chain—Horizontal method for the detection of *Cronobacter* spp.

ThermoFisher SCIENTIFIC

The initial certification study and all subsequent extension studies were conducted by ADRIA Développement, Quimper, France.

The protocols for the alternative method and the reference method are summarized in Appendix 1 and 2, respectively.

Inclusivity & exclusivity study

A total of 57 inclusivity isolates of *Cronobacter* species including; *C. dublinensis, C. malonaticus, C. muytjensii, C. sakazakii, C. dublinensis lactaridi, C. dublinensis, C. lausannensis, C. turicencis, C. universalis* and *C. condimenti,* and 31 exclusivity isolates were analyzed.

Inclusivity and exclusivity isolates were cultured in Thermo Scientific™ Oxoid™ Brain Heart Infusion (BHI) Broth. The inclusivity BHI cultures were inoculated into Thermo Scientific™ Buffered Peptone Water (BPW) (ISO) supplemented with 6 mg/L vancomycin at a level of 10 CFU/90 mL. The exclusivity cultures were inoculated into BPW (ISO) without novobiocin at a level of 10^5 CFU/mL.

The inclusivity and exclusivity enrichments were then analyzed following the alternative method protocol (Appendix 1).

Inclusivity & exclusivity results

The alternative method successfully returned positive results for all 57 inclusivity isolates and correctly identified all 31 exclusivity isolates as negative; demonstrating the alternative method is a sensitive and specific method.

Inter-laboratory study

An inter-laboratory study was performed as part of the initial validation. A matrix of PIF with probiotics was prepared and spiked with a *Cronobacter sakazakii* isolate and sent to all participating laboratories. To facilitate the testing, the PIF sample was first homogenized in sterile water. Samples were analyzed following both the alternative method and the ISO reference method (Appendix 1 and 2, respectively). Of all the samples tested, one third were unspiked, one third were spiked with a low level inoculum (1 CFU/10 g) and the remaining third were spiked with a high level inoculum (10 CFU/10 g).

Table 1. Inter-laboratory study result summary

Sensitivity for the alternative method	91.7%
Sensitivity for the reference method	88.9%
Relative trueness	89.9%
False positive ratio	0.0%

Inter-laboratory study results

The results displayed in Table 1 demonstrate the alternative method is reliable for the detection of *Cronobacter* species from PIF and production environment samples achieving 100% sensitivity and a false positive ratio of 0.0%.

Method comparison study

A total of 210 samples (consisting of 64 10 g PIF samples, 69 production environment samples and 77 300 g PIF samples) were analyzed using the Applied Biosystems 7500 Fast Food Safety System. The results are presented in Table 2.

A total of 208 lysates (consisting of 64 10 g PIF samples, 67 production environment samples and 77 300 g PIF samples) were analyzed using the Applied Biosystems QuantStudio 5 Food Safety System. The results are presented in Table 3.

Method comparison study results

Table 2. Sensitivity results when using the Applied Biosystems 7500 Fast Food Safety System

Category	PA	NA	PD	ND	PPND	PPNA	Sensitivity of the Alternative Method	Sensitivity of the Reference Method	Relative Trueness	False Positive Ratio
PIF 10 g	29	32	1	0	0	2	100.0	96.7	98.4	5.9
Production environment samples	24	38	4	3	0	0	90.3	87.1	89.9	0.0
PIF 300 g	23	39	9	5	0	1	86.5	75.7	81.8	2.6
Total	**76**	**109**	**14**	**8**	**0**	**3**	**91.8**	**85.7**	**89.5**	**2.7**

PA = Positive agreement PD = Positive deviation NA = Negative agreement ND = Negative deviation
PPND = Predictive positive negative deviation PPNA = Predictive positive negative agreement

Table 3: Sensitivity results when using the Applied Biosystems QuantStudio 5 Food Safety System

Category	PA	NA	PD	ND	PPND	PPNA	Sensitivity of the Alternative Method	Sensitivity of the Reference Method	Relative Trueness	False Positive Ratio
PIF 10 g	29	32	1	0	0	2	100.0	96.7	98.4	5.9
Production environment samples	22	37	4	3	0	1	89.7	86.2	89.6	2.6
PIF 300 g	23	38	9	3	2	2	86.5	75.7	81.8	10.5
Total	**74**	**107**	**14**	**6**	**2**	**5**	**91.7**	**85.4**	**89.4**	**6.3**

PA = Positive agreement PD = Positive deviation NA = Negative agreement ND = Negative deviation
PPND = Predictive positive negative deviation PPNA = Predictive positive negative agreement

Eight negative deviations (ND and PPND) were observed when using both the Applied Biosystems 7500 Fast and Applied Biosystems QuantStudio 5 Food Safety Systems. The confirmation results were negative for all ND and PPND samples. The negative deviations are likely due to the unpaired study design and the related sampling heterogeneity. As *Cronobacter* species could not be isolated from the samples by the culture confirmation method, it is likely that no target cells were present in the portion of matrix used for the alternative method.

Fourteen positive deviation (PD) results were recorded when using both the Applied Biosystems 7500 Fast and Applied Biosystems QuantStudio 5 Food Safety Systems. The confirmation results were positive for all PD and PPNA samples. This showed that 14 results were correctly detected as positive using the alternative method, but failed to be detected with the reference method.

The method comparison study results demonstrate that the alternative method showed equivalent or improved sensitivity performance to the ISO 22964:2017 reference method when using the Applied Biosystems 7500 Fast Food Safety System and the Applied Biosystems QuantStudio 5 Food Safety System.

Relative level of detection study

For the relative level of detection (RLOD) study, four individual *Cronobacter* species isolates were spiked into four matrices and analyzed using the Applied Biosystems 7500 Fast Food Safety System.

The lysates were no longer available for analysis on the Applied Biosystems QuantStudio 5 Food Safety System, therefore only lysates from one matrix/strain pair were tested in agreement with the AFNOR Technical Committee.

The RLOD samples were analyzed using the ISO reference method detailed in ISO 22964:2017 prior to inoculation to verify the absence of *Cronobacter* species. After inoculation, samples were tested using the ISO reference method and the alternative method. Table 4 outlines the matrices and the inoculated strains for each Food Safety System.

Table 4: Defined (matrix/strain) pairs for the RLOD study

Matrix	Inoculated strain	Food safety system
PIF without probiotics (10 g)	*Cronobacter sakazakii* Ad1418	7500 Fast
PIF with probiotics (10 g)	*Cronobacter sakazakii* Ad1418	7500 Fast
Process water	*Cronobacter turicensis* Ad1445	7500 Fast
PIF with probiotics (300 g)	*Cronobacter sakazakii* Ad1446	7500 Fast QuantStudio 5

Relative level of detection results

The RLOD for each Food Safety System was calculated using the Excel™ spreadsheet available at http://standards.iso.org/iso/16140 - RLOD (clause 5-1-4-2 Calculation and interpretation of RLOD) version 06.07.2015. The results are displayed in Table 5.

Table 5: RLOD study results

Matrix	Inoculated strain	Food safety system	RLOD	Acceptability limit (≤)
PIF without probiotics (10 g)	*Cronobacter sakazakii* Ad1418	7500 Fast	1.000	2.5
PIF with probiotics (10 g)	*Cronobacter sakazakii* Ad1418	7500 Fast	1.148	
Process water	*Cronobacter turicensis* Ad1445	7500 Fast	1.038	
PIF with probiotics (300 g)	*Cronobacter sakazakii* Ad1446	7500 Fast	1.482	
Combined RLOD for 7500 Fast			**1.166**	

As shown in Table 5, the combined RLOD result for the Applied Biosystem 7500 Fast Food Safety System is 1.166 and the RLOD result for the Applied Biosystem QuantStudio 5 Food Safety system is 1.482, which is below the acceptability limit of ≤2.5 for an unpaired study.

Conclusion

The NF VALIDATION studies demonstrate that the SureTect Cronobacter species PCR Assay workflow is superior or equivalent in performance to the ISO reference method detailed in ISO 22964:2017 for the detection of *Cronobacter* spp. from PIF (10 g and 300 g) and production environment samples when using the Applied Biosystems 7500 Fast or the Applied Biosystems QuantStudio 5 Food Safety Systems.

In addition, the SureTect Cronobacter species PCR Assay (PT1060A) and the Thermo Scientific™ SureTect™ Salmonella species PCR Assay (PT0100A) can be utilized from the same enrichment when testing PIF 10 g samples due to the paired enrichment protocol.

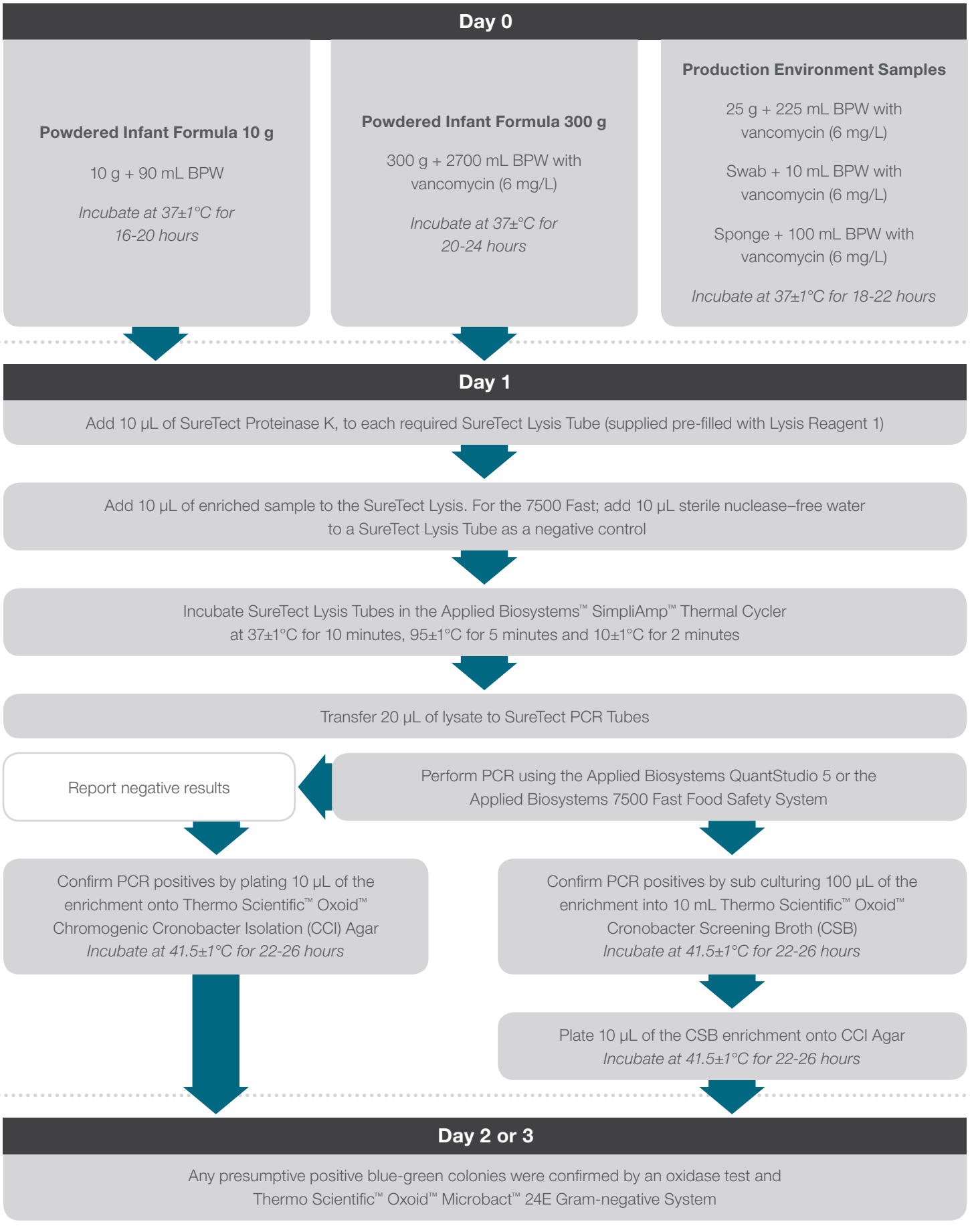
The NF VALIDATION certificate is available from www.thermofisher.com/foodsafety

Contact Information:
microbiology@thermofisher.com
USA +1 800 255 6730
International +44 (0) 1256 84114

LT2437A
September 2019

ThermoFisher SCIENTIFIC

Appendix 1. Workflow for the alternative method when using the Applied Biosystems QuantStudio 5 Food Safety System



Appendix 2: Workflow for the ISO reference method: ISO 22964 (April 2017) - Microbiology of the food chain - Horizontal method for the detection of *Cronobacter* spp.

Day 0

Powdered Infant Formula 10 g

10 g + 90 mL BPW

Incubate at 34-38°C for 18±2 hours

Production environment samples

10 g or 10 mL + 90 mL BPW

Swab + 10 mL BPW

Sponge + 100 mL BPW

Incubate at 34-38°C for 18±2 hours

Day 1

Add 0.1 mL to 10 mL CSB

Incubate for 24±2 hours at 41.5±1°C

Day 2

Streak 10 µL onto a CCI Agar plate

Incubate for 24±2 hours at 41.5±1°C

Day 3

Perform confirmatory tests on one typical colony and four other colonies.
If the first colony is negative, streak 10 µL onto Thermo Scientific™ Oxoid™ Tryptone Soya Agar.

Incubate at 37±°C for 18-24 hours

Day 4

Perform biochemical confirmation