Study Report

Thermo Scientific SureTect Cronobacter species PCR Assay method (using the Thermo Scientific PikoReal Real-Time PCR Instrument), NF VALIDATION ISO 16140: Relative Limit of Detection

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Relative Limit of Detection

As part of the NF VALIDATION™ ISO 16140 validation, a study to determine the relative limit of detection of the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay method (PT1060A) against the ISO Technical Specification detailed in ISO/TS 22964:2006 was performed. Results showed that the SureTect Cronobacter species PCR Assay method is a suitable alternative method for the detection of low levels of *Cronobacter* species from powdered infant formula (with and without probiotics) and production environment samples.

Methodology

Choice of strains: Isolates were selected from the culture collection at ADRIA Développement, Quimper, France and were spiked into samples from two of the categories validated in this study

1. Supplemented ("probiotic") and unsupplemented powdered infant formula
2. Production environment samples

Samples were prepared using the strains detailed in Table 1 to produce three batches of each matrix which consisted of one uninoculated sample set, one sample set inoculated at ~4 CFU/10g to give fractional positive results across 20 samples and one sample set of five replicates inoculated at 8 CFU/10g to assess the relative limit of detection (RLOD).

Table 1: Defined Matrix-Strain Pairs for RLOD Determination

Matrix	Isolate	Storage condition before RLOD analysis
Powdered infant formula with probiotic supplements	*Cronobacter sakazakii* Ad1418	2 weeks at room temperature
Powdered infant formula without probiotics supplements	*Cronobacter sakazakii* Ad 1418	
Process water	*Cronobacter sakazakii* Ad 1445	4°C, 48-72 h

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Sample preparation: 10g of each sample was homogenized with 90ml of either BPW (ISO) for infant formula samples or BPW (ISO) supplemented with 6mg/l vancomycin for environmental samples. Samples were incubated at 37 ±1°C, with powdered infant formula samples being incubated for 16-20h and process water samples for 18-22h before analysis with the SureTect Cronobacter species Assay method.

Method: 10µl of SureTect Proteinase K Reagent was added to each of the prefilled SureTect Lysis Tubes. Next, 10µl of the enriched sample was added to the Lysis Tubes, which were then heated at 37 ±1°C for 10 minutes, followed by 95 ±1°C for 5 min. The tubes were cooled by leaving in a rack at room temperature and 20µl aliquots of the lysates were transferred to SureTect PCR Tubes containing PCR tablets that were then immediately transferred to the Thermo Scientific™ PikoReal™ Real-time PCR Instrument for processing. All samples, regardless of the PCR result were confirmed by plating 10µl of the BPW enrichments onto Chromogenic Cronobacter Isolation (CCI) Agar, which was incubated at 41.5 ±1°C for 22-26h and any presumptive positive blue-green colonies were confirmed using a biochemical micro-gallery kit.

Reference method protocol

10g of each sample was analyzed using the method detailed in ISO/TS 22964:2006. Each sample was enriched in 90ml of BPW (ISO) and incubated at 37 ±1°C for 16-20h. Following incubation 100µl of the enrichment was sub-cultured into 10ml modified Lauryl Sulphate Tryptone Broth (mLST) supplemented with 10mg/l vancomycin and incubated for 22-26 h at 44 ±0.5°C. 10µl of the mLST Broth enrichment was then streaked onto Enterobacter sakazakii Isolation Agar, which was then incubated at 44 ±1°C for 22-26 hours. Presumptive positive, blue-green colonies were streaked to Tryptone Soya Agar, which was then incubated at 25 ±1°C for 44-48 h. Yellow colonies on TSA were confirmed as *Cronobacter* species by oxidase and biochemical identification tests.

Table 2: RLOD Results for the SureTect and ISO/TS Methods

Matrix	Isolate	Relative level of detection for the SureTect Cronobacter species PCR Kit (CFU/10g)
Powdered infant formula with probiotics	*Cronobacter sakazakii* Ad1418	0.568 [0.254-1.269]
Powdered infant formula without probiotics	*Cronobacter sakazakii* Ad 1418	1.000 [0.478-2.092]
Process water	*Cronobacter turicensis* Ad 1445	1.038 [0.449-2.399]

Conclusion

The SureTect Cronobacter species PCR Assay method meets the ISO16140:2015 acceptability criteria for relative limit of detection (RLOD) performance and demonstrated an excellent level of detection during the validation study. The limit of detection range was demonstrated to be 0.2-1.2 cells per 10g of probiotic infant formula and 0.4-2.0 cells per 10g of non-probiotic infant formula after an enrichment time of 16 hours. The limit of detection range from process water was demonstrated to be 0.4-2.3 cells per 10ml after 18 hours incubation, proving that this SureTect method is an accurate alternative method for the detection of *Cronobacter* species from powdered infant formula (with and without probiotics) and production environmental samples.

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