STUDY REPORT

SureTect Cronobacter species PCR Assay Workflow NF VALIDATION ISO 16140 – Extension study: Relative Level of Detection

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Summary

As part of the NF VALIDATION™ ISO 16140 extension study of the Thermo Scientific™ SureTect™ Cronobacter species PCR Assay workflow (alternative method), a method comparison study was conducted by ADRIA Développement, Quimper, France. The alternative method has previously been validated for 10 g powdered Infant Formula (PIF) samples and production environment samples with the Thermo Scientific™ SureTect™ PikoReal™ Real-Time PCR Instrument with Thermo Scientific™ SureTect™ version 1.2 software. The extension study was designed to validate the use of the alternative method with the SureTect PikoReal Instrument software for 300 g PIF, and the Applied Biosystems™ 7500 Fast Real-Time PCR System and Applied Biosystems™ Rapid Finder™ Express version 2.0 software for 10 g and 300 g PIF, and production environment samples. The following is a summary of the relative level of detection study.

Methodology

Choice of strains:

Cronobacter isolates from the culture collection at ADRIA Développement were spiked into each of four different matrices (PIF without probiotics (10 g sample size), PIF with probiotics (10 g and 300 g sample size), and process water) analyzed during the NF VALIDATION ISO 16140 relative level of detection study.

Sample preparation:

- The 10 g PIF samples were homogenized with 90 ml of BPW (ISO) and enriched by incubating at 37±1 °C for 16-20 hours.
- The 300 g PIF samples were homogenized with 2700 ml of BPW (ISO) supplemented with 6 mg/ml vancomycin and enriched by incubating at 37±1 °C for 20-24 hours.
- The production environment samples were homogenized with 90 ml of BPW (ISO) supplemented with 6 mg/l vancomycin and enriched by incubating at 37±1 °C for 18-22 hours.

The enriched samples were then analyzed with the alternative method.

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Alternative method:
Ten microlitres of SureTect Proteinase K reagent were added to the required number of SureTect Lysis Tubes (supplied pre-filled with Lysis Reagent 1) before adding 10 µl of the enrichments to the Lysis Tubes, which were then heated at 37±1 °C for 10 minutes, followed by 95±1 °C for 5 minutes. The tubes were cooled by leaving in a rack at room temperature for around 2 minutes and 20 µl aliquots of the lysates were transferred to SureTect PCR Tubes containing SureTect Cronobacter spp. PCR tablets.

When performing PCR using the Applied Biosystems 7500 Fast System, a negative control sample was prepared by adding 10 µl sterile nuclease free water (or sterile medium to a SureTect Lysis Tube, instead of the enriched sample.

The SureTect PCR Tubes were then immediately transferred to the SureTect PikoReal Instrument or the Applied Biosystems 7500 Fast System for processing. All samples, regardless of the PCR result were confirmed by plating 10 µl of the BPW enrichments onto Chromogenic Cronobacter Isolation (CCI) Agar, which was incubated at 41.5±1 °C for 22-26 hours and any presumptive positive blue-green colonies were confirmed using a Microbact™ 24E biochemical micro-gallery kit.

ISO reference method:
Ten grams of each sample were analyzed using the method detailed in ISO/TS 22964:2006. Each sample was enriched in 90 ml of BPW (ISO) and incubated at 37±1 °C for 16-20 hours. Following incubation, 100 µl of the enrichment were sub-cultured into 10 ml modified Lauryl Sulphate Tryptone Broth (mLST) supplemented with 10 mg/l vancomycin and incubated for 22-26 hours at 44±0.5 °C. Ten microlitres of the mLST Broth enrichment were then streaked onto Enterobacter sakazakii Isolation Agar (ESIA), 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 hours. Yellow colonies on TSA were confirmed as Cronobacter species by oxidase test, as well as confirmation using a Thermo Scientific™ Microbact™ GNB 24E (Thermo Fisher Scientific) biochemical micro-gallery kit.

Results

The relative levels of detection for the alternative method using the SureTect PikoReal instrument and the Applied Biosystems 7500 Fast PCR System were determined according to ISO 16140-2:2016. The relative levels of detection were analyzed according to the Spearman-Kärber (LOD50) method (Table 1) to give the relative levels of detection for each strain-matrix combination analyzed during the AFNOR Certification validation study.

Table 1: RLOD Results for the alternative method

Matrix/strain pairs	Incubation time	Relative level of detection for the SureTect Cronobacter species Assay	
		SureTect PikoReal PCR Instrument	Applied Biosystems 7500 Fast PCR System
PIF without probiotic supplements (10 g)/Cronobacter sakazakii Ad1418	16-20 hours	0.628 [0.279 – 1.416]	0.513 [0.231 – 1.139]
PIF with probiotic supplements (10 g)/Cronobacter sakazakii Ad1418	16-20 hours	1.000 [0.478 – 2.092]	1.000 [0.478 – 2.092]
PIF with probiotic supplements (300 g)/Cronobacter sakazakii Ad1446	16-20 hours	1.206 [0.566 – 2.570]	1.206 [0.566 – 2.570]
Process production samples Cronobacter turicensis Ad1445	16-20 hours	1.038 [0.449 – 2.399]	1.038 [0.449 – 2.399]

Conclusion

The relative level of detection study conducted as part of the NF VALIDATION extension study demonstrated that the level of detection of the SureTect Cronobacter species PCR Assay workflow met the acceptability limits for an unpaired study, as detailed in the ISO/TS 22964:2006, using both the Thermo Scientific SureTect PikoReal PCR instrument (0.3-2.6 level of detection) and the Applied Biosystems 7500 Fast System (0.5-2.5 level of detection). The NF VALIDATION certificate and validation report summary for this study are available from **http://nf-validation.afnor.org/en/.**

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