STUDY REPORT

# SureTect Cronobacter species PCR Assay Workflow NF VALIDATION ISO 16140 – Extension study: Inclusivity and Exclusivity

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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 inclusivity and exclusivity part of the study.

## Methodology

Choice of strains

Fifty seven inclusivity isolates of *Cronobacter* spp. and 31 exclusivity isolates were analyzed as part of the NF VALIDATION by AFNOR Certification ISO 16140 validation study.

Culture enrichment

Each inclusivity isolate was cultured in Brain Heart Infusion (BHI) Broth. Prepared cultures were diluted in Peptone Salt Solution and inoculated at approximately 10-100 CFU/225 ml of Buffered Peptone Water (BPW) (ISO) supplemented with 6 mg/l vancomycin and incubated at 37±1 °C for 16-20 hours. Once incubated, an aliquot of the enrichment was analyzed according to the alternative method.

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Each exclusivity isolate was cultured in BHI Broth. Prepared cultures were diluted in Buffered Peptone Water (ISO) to obtain approximately 1x$10^5$ CFU. Prepared dilutions were incubated for at 37±1 °C for 22-26 hours, prior to analyzing with the alternative method.

## Protocol

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 media) to a SureTect Lysis Tube, instead of the enriched sample.

The PCR Tubes were then immediately transferred to the SureTect PikoReal Instrument or the Applied Biosystems 7500 Fast System for processing.

## Results

Inclusivity and exclusivity results are summarized in tables 1 and 2 respectively. All 57 inclusivity isolates tested returned a PCR positive result.

All 31 exclusivity isolates gave negative results with the alternative method.

**Table 1: NF VALIDATION ISO 16140 Inclusivity results for the alternative method**

| Isolate | ID | Result | |
|---|---|---|---|
| | | SureTect PikoReal Instrument | Applied Biosystems 7500 Fast System |
| *Cronobacter dublinensis* | DSM18705 | Positive | Positive |
| *Cronobacter malonaticus* | DSM18702 | Positive | Positive |
| *Cronobacter malonaticus* | Ad1708 | Positive | Positive |
| *Cronobacter muytjensii* | CIP103581 | Positive | Positive |
| *Cronobacter sakazakii* | Ad939 | Positive | Positive |
| *Cronobacter sakazakii* | Ad940 | Positive | Positive |
| *Cronobacter sakazakii* | Ad941 | Positive | Positive |
| *Cronobacter sakazakii* | Ad942 | Positive | Positive |
| *Cronobacter sakazakii* | Ad943 | Positive | Positive |
| *Cronobacter sakazakii* | Ad944 | Positive | Positive |
| *Cronobacter sakazakii* | Ad945 | Positive | Positive |
| *Cronobacter sakazakii* | Ad946 | Positive | Positive |
| *Cronobacter sakazakii* | Ad947 | Positive | Positive |
| *Cronobacter sakazakii* | Ad948 | Positive | Positive |
| *Cronobacter sakazakii* | Ad949 | Positive | Positive |
| *Cronobacter sakazakii* | Ad950 | Positive | Positive |
| *Cronobacter sakazakii* | Ad951 | Positive | Positive |
| *Cronobacter sakazakii* | Ad952 | Positive | Positive |
| *Cronobacter sakazakii* | Ad953 | Positive | Positive |

**Table 1 (continued): NF VALIDATION ISO 16140 Inclusivity results for the alternative method**

| Isolate | ID | Result | |
|---|---|---|---|
| | | SureTect PikoReal Instrument | Applied Biosystems 7500 Fast System |
| *Cronobacter sakazakii* | Ad963 | Positive | Positive |
| *Cronobacter sakazakii* | Ad704 | Positive | Positive |
| *Cronobacter sakazakii* | Ad831 | Positive | Positive |
| *Cronobacter sakazakii* | Ad829 | Positive | Positive |
| *Cronobacter sakazakii* | Ad916 | Positive | Positive |
| *Cronobacter sakazakii* | Ad898 | Positive | Positive |
| *Cronobacter dublinensis lactaridi* | DSMZ18707T | Positive | Positive |
| *Cronobacter dublinensis lausannensis* | DSMZ18706T | Positive | Positive |
| *Cronobacter sakazakii* | Ad1418 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1419 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1420 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1421 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1424 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1425 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1426 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1427 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1428 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1429 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1430 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1431 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1432 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1433 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1434 | Positive | Positive |
| *Cronobacter sakazakii* | Ad1435 | Positive | Positive |
| *Cronobacter turicensis* | Ad1445 | Positive | Positive |
| *Cronobacter turicensis* | DSMZ18703 | Positive | Positive |
| *Cronobacter malonaticus* | E752 | Positive | Positive |
| *Cronobacter turicensis* | E681 | Positive | Positive |
| *Cronobacter muytjensii* | E769 | Positive | Positive |
| *Cronobacter sakazakii* | Ad893 | Positive | Positive |
| *Cronobacter sakazakii* | Ad894 | Positive | Positive |
| *Cronobacter sakazakii* | Ad895 | Positive | Positive |

**Table 1 (continued): NF VALIDATION ISO 16140 Inclusivity results for the alternative method**

| Isolate | ID | Result | |
|---|---|---|---|
| | | SureTect PikoReal Instrument | Applied Biosystems 7500 Fast System |
| *Cronobacter sakazakii* | Ad896 | Positive | Positive |
| *Cronobacter sakazakii* | Ad897 | Positive | Positive |
| *Cronobacter dublinensis* subsp *dublinensis* | LMG 23823T | Positive | Positive |
| *Cronobacter dublinensis* subsp *lausannensis* | E798 | Positive | Positive |
| *Cronobacter universalis* | NCTC 9529T | Positive | Positive |
| *Cronobacter condimenti* | LMG 26250T | Positive | Positive |

**Table 2: NF VALIDATION ISO 16140 Exclusivity results for the alternative method**

| Isolate | ID | Result | |
|---|---|---|---|
| | | SureTect PikoReal Instrument | Applied Biosystems 7500 Fast System |
| *Citrobacter braaki* | Ad833 | Negative | Negative |
| *Citrobacter diversus* | Ad173 | Negative | Negative |
| *Citrobacter farmeri* | Ad116 | Negative | Negative |
| *Citrobacter freundii* | 39 | Negative | Negative |
| *Citrobacter koseri* | CIP105177 | Negative | Negative |
| *Enterobacter aerogenes* | Ad889 | Negative | Negative |
| *Enterobacter agglomerans* | 11 | Negative | Negative |
| *Enterobacter agglomerans* | 136 | Negative | Negative |
| *Enterobacter amnigenus* | 52 | Negative | Negative |
| *Enterobacter amnigenus* | 129 | Negative | Negative |
| *Enterobacter amnigenus* | A00C068 | Negative | Negative |
| *Enterobacter cloacae* | 51 | Negative | Negative |
| *Enterobacter cloacae* | 10 | Negative | Negative |
| *Enterobacter fergusonii* | 2876 | Negative | Negative |
| *Enterobacter gergoviae* | CIP76.1 | Negative | Negative |
| *Enterobacter helveticus* | DSM 18396 | Negative | Negative |
| *Enterobacter hormaechei* | Ad990 | Negative | Negative |
| *Enterobacter intermedius* | 60 | Negative | Negative |
| *Enterobacter kobei* | Ad706 | Negative | Negative |
| *Escherichia coli* | 16 | Negative | Negative |

**Table 2 (continued): NF VALIDATION ISO 16140 Exclusivity results for the alternative method**

| Isolate | ID | Result | |
|---|---|---|---|
| | | SureTect PikoReal Instrument | Applied Biosystems 7500 Fast System |
| *Escherichia hermanii* | Ad462 | Negative | Negative |
| *Hafnia alvei* | Ad245 | Negative | Negative |
| *Klebsiella pneumoniae* | 122 | Negative | Negative |
| *Leclercia adecarboxylata* | Ad707 | Negative | Negative |
| *Salmonella arizonae (51:z4,z23)* | CIP 5523 | Negative | Negative |
| *Salmonella diarizonae SIIIb 65:c:z* | Ad 1298 | Negative | Negative |
| *Salmonella Typhimurium* | Ad1333 | Negative | Negative |
| *Serratia ficaria* | 113 | Negative | Negative |
| *Serratia marcescens* | Ad455 | Negative | Negative |
| *Yersinia intermediai* | Ad133 | Negative | Negative |
| *Escherichia coli* O103 | Ad 1862 | Negative | Negative |

## Conclusion

The inclusivity and exclusivity study conducted as part of this NF VALIDATION ISO 16140 extension study demonstrated that the SureTect Cronobacter species workflow showed 100% inclusivity and 100% exclusivity, when using both SureTect PikoReal Instrument and the Applied Biosystems 7500 Fast System. The NF VALIDATION certificate and a summary of the expert laboratory report of this study are available from **http://nf-validation.afnor.org/en/.**

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