

# Thermo Scientific SureTect Cronobacter species Assay (using the Thermo Scientific PikoReal Real-Time PCR instrument) NF VALIDATION ISO 16140: Inclusivity and exclusivity

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## Inclusivity and exclusivity

As part of the NF VALIDATION™ ISO 16140 regulatory validation study of the Thermo Scientific™ SureTect™ Cronobacter species Assay method, an inclusivity and exclusivity study was conducted by ADRIA Développement, Quimper, France. Results showed that the SureTect Cronobacter species Assay method was equivalent in performance to the ISO reference method (ISO/TS 22964:2006). The following is a summary of the inclusivity and exclusivity part of the study.

### Methodology

**Choice of strains:** Fifty-seven inclusivity isolates of *Cronobacter* species: *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. sakazakii*, *C. dublinensis lactaridi*, *C. dublinensis lausannensis*, *C. turicensis*, *C. universalis* and *C. condimentii*, and thirty-one exclusivity isolates were analyzed as part of the NF VALIDATION by AFNOR Certification ISO 16140 validation study.

**Culture enrichment:** For each inclusivity isolate, one colony was inoculated into a tube containing Brain Heart Infusion (BHI) Broth and incubated for 24 hours at 37°C. The cultures were then decimally diluted using Peptone Salt Solution and inoculated at approximately 10 CFU/90ml of Buffered Peptone Water (BPW) (ISO) supplemented with 6mg/l vancomycin and incubated for 24 h at 37°C. Once incubated, an aliquot of the enrichment was analyzed according to the SureTect method protocol.

Each of the exclusivity isolates were incubated into BHI Broth. Prepared cultures were diluted in Buffered Peptone Water (ISO) to obtain approximately 10<sup>5</sup> CFU/ml. Prepared dilutions were incubated for 24 h at 37°C before analyzing according to the SureTect method protocol.

**Method:** 10µl of SureTect Proteinase K reagent was added to each of the required number of SureTect Lysis Tubes (prefilled with Lysis Reagent 1). 10µl of the sample prepared as detailed above was added to the Lysis Tubes, which were then heated at 37°C for 10 minutes, followed by 95°C for 5 min. The tubes were cooled by leaving in a rack at room temperature and then 20µl aliquots of the lysates were transferred to SureTect PCR Tubes containing PCR tablets which were then immediately transferred to the Thermo Scientific™ SureTect™ PikoReal™ Real-time PCR Instrument for processing.

## Results

Inclusivity and exclusivity results are summarised in Tables 1 and 2 respectively. All 57 inclusivity isolates tested returned positive results and all of the exclusivity isolates gave negative results with the SureTect Assay method.

Table 1: NF VALIDATION ISO 16140  
Inclusivity results for the SureTect  
Cronobacter species Assay method

Isolate	ID	Result	Isolate	ID	Result
<i>C. dublinensis</i>	DSM18705	Positive	<i>C. sakazakii</i>	Ad898	Positive
<i>C. malonaticus</i>	DSM18702	Positive	<i>C. dublinensis lactandi</i>	DSMZ18707T	Positive
<i>C. malonaticus</i>	Ad1708	Positive	<i>C. dublinensis lausannensis</i>	DSMZ18706T	Positive
<i>C. muytjensii</i>	CIP103581	Positive	<i>C. sakazakii</i>	Ad1418	Positive
<i>C. sakazakii</i>	Ad939	Positive	<i>C. sakazakii</i>	Ad1419	Positive
<i>C. sakazakii</i>	Ad940	Positive	<i>C. sakazakii</i>	Ad1420	Positive
<i>C. sakazakii</i>	Ad941	Positive	<i>C. sakazakii</i>	Ad1421	Positive
<i>C. sakazakii</i>	Ad942	Positive	<i>C. sakazakii</i>	Ad1424	Positive
<i>C. sakazakii</i>	Ad943	Positive	<i>C. sakazakii</i>	Ad1425	Positive
<i>C. sakazakii</i>	Ad944	Positive	<i>C. sakazakii</i>	Ad1426	Positive
<i>C. sakazakii</i>	Ad945	Positive	<i>C. sakazakii</i>	Ad1427	Positive
<i>C. sakazakii</i>	Ad946	Positive	<i>C. sakazakii</i>	Ad1428	Positive
<i>C. sakazakii</i>	Ad947	Positive	<i>C. sakazakii</i>	Ad1429	Positive
<i>C. sakazakii</i>	Ad948	Positive	<i>C. sakazakii</i>	Ad1430	Positive
<i>C. sakazakii</i>	Ad949	Positive	<i>C. sakazakii</i>	Ad1431	Positive
<i>C. sakazakii</i>	Ad950	Positive	<i>C. sakazakii</i>	Ad1432	Positive
<i>C. sakazakii</i>	Ad951	Positive	<i>C. sakazakii</i>	Ad1433	Positive
<i>C. sakazakii</i>	Ad952	Positive	<i>C. sakazakii</i>	Ad1434	Positive
<i>C. sakazakii</i>	Ad953	Positive	<i>C. sakazakii</i>	Ad1435	Positive
<i>C. sakazakii</i>	Ad963	Positive	<i>C. turicensis</i>	Ad1445	Positive
<i>C. sakazakii</i>	Ad704	Positive	<i>C. turicensis</i>	DSMZ18703	Positive
<i>C. sakazakii</i>	Ad831	Positive	<i>C. malonaticus</i>	E752	Positive
<i>C. sakazakii</i>	Ad829	Positive	<i>C. turicensis</i>	E681	Positive
<i>C. sakazakii</i>	Ad916	Positive	<i>C. muytjensii</i>	E769	Positive
<i>C. sakazakii</i>	Ad893	Positive	<i>C. dublinensis subsp. dublinensis</i>	LMG23823T	Positive
<i>C. sakazakii</i>	Ad894	Positive	<i>C. dublinensis subsp. lausanensis</i>	E798	Positive
<i>C. sakazakii</i>	Ad895	Positive	<i>C. universalis</i>	NCTC 9529T	Positive
<i>C. sakazakii</i>	Ad896	Positive	<i>C. condimenti</i>	LMG26250T	Positive
<i>C. sakazakii</i>	Ad897	Positive			

Table 2: NF VALIDATION ISO 16140  
Exclusivity Results for the SureTect  
Salmonella species Assay method

Isolate	ID	Result	Isolate	TCC No	Result
<i>Citrobacter braakii</i>	Ad833	Negative	<i>Enterobacter hormaechei</i>	Ad990	Negative
<i>Citrobacter diversus</i>	Ad173	Negative	<i>Enterobacter intermedius</i>	60	Negative
<i>Citrobacter fameri</i>	Ad116	Negative	<i>Enterobacter kobei</i>	Ad706	Negative
<i>Citrobacter freundii</i>	39	Negative	<i>Escherichia coli</i>	16	Negative
<i>Citrobacter koseri</i>	CIP105177	Negative	<i>Escherichia hermannii</i>	Ad462	Negative
<i>Enterobacter aerogenes</i>	Ad889	Negative	<i>Hafnia alvei</i>	Ad245	Negative
<i>Enterobacter agglomerans</i>	11	Negative	<i>Klebsiella pneumoniae</i>	122	Negative
<i>Enterobacter agglomerans</i>	136	Negative	<i>Leclercia adecarboxylata</i>	Ad707	Negative
<i>Enterobacter amnigenus</i>	52	Negative	<i>Salmonella arizoniae</i> 51:z4:z23	CIP5523	Negative
<i>Enterobacter amnigenus</i>	129	Negative	<i>Salmonella diarizoniae</i> 65:c:z	Ad1298	Negative
<i>Enterobacter amnigenus</i>	A00C068	Negative	<i>Salmonella Typhimurium</i>	Ad1333	Negative
<i>Enterobacter cloacae</i>	51	Negative	<i>Serratia ficaria</i>	113	Negative
<i>Enterobacter cloacae</i>	10	Negative	<i>Serratia marcescens</i>	Ad455	Negative
<i>Enterobacter fergusonii</i>	2876	Negative	<i>Yersinia intermedia</i>	Ad133	Negative
<i>Enterobacter gergoviae</i>	CIP76.1	Negative	<i>Escherichia coli</i> O103	Ad1862	Negative
<i>Enterobacter helveticus</i>	DSM18396T	Negative			

### Conclusions

The inclusivity and exclusivity study conducted as part of this NF VALIDATION ISO 16140 validation study demonstrated that the SureTect Cronobacter species PCR Assay method showed 100% inclusivity and 100% exclusivity with the isolates analyzed.

[thermoscientific.com/SureTect](http://thermoscientific.com/SureTect)

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