



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
092401

The AOAC Research Institute hereby certifies the method known as:

Thermo Scientific™ SureTect™ Cronobacter species PCR Assay

manufactured by
Oxoid Ltd part of Thermo Fisher Scientific
Wade Rd
Basingstoke, Hampshire
RG24 8PW, UK

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*SM Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink, appearing to read 'Bradley A. Stawick'.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date
Expiration Date

September 19, 2024
December 31, 2024

METHOD NAME

Thermo Scientific™ SureTect™ Cronobacter species PCR Assay

CATALOG NUMBER

A56845

ORIGINAL CERTIFICATION DATE

September 11, 2024

PRINCIPLE OF THE METHOD

The SureTect Cronobacter PCR Assay method is used in conjunction with either the Applied Biosystems™ QuantStudio™ 5 Real-Time Food Safety PCR instrument with Thermo Scientific™ RapidFinder™ Analysis Software (version 3.0 or higher) or the Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with Applied Biosystems™ RapidFinder™ Express Software (version 2.0 or higher) for the detection of *Cronobacter* spp. in PIF and related ingredients and environmental samples.

The SureTect Cronobacter PCR Assay is based upon the use of Solaris™ reagents for performing PCR. Dye-labelled probes target unique DNA sequences specific to *Cronobacter* spp. and an internal positive control (IPC). Target DNA, if present, is detected by real-time PCR. Analysis software provides interpretation of results. Analysis software provides interpretation of results. The assay includes an internal positive control (IPC) for each reaction to confirm that the PCR process has occurred. It is unnecessary to incorporate positive control organisms with routine testing of samples.

Enriched food and environmental samples are combined directly with ready-to-use Lysis Reagent 1 and Proteinase K to lyse bacterial cells present in the sample and release their DNA into solution. Lysates are transferred to the Cronobacter species PCR Tubes to rehydrate the lyophilized PCR pellets. The pellets contain lyophilized target-specific primers, dye-labelled probes, and PCR master mix components. The PCR tubes are sealed, loaded into the real-time PCR instrument, then the run is started using the RapidFinder software. After the run is complete, the software displays the interpreted results as simple positive or negative symbols. The results can be reported, stored, printed, and downloaded as required. Results are achieved approximately 80 min after loading the prepared sample into the instrument.

CERTIFIED CLAIM STATEMENT: The Thermo Scientific™ SureTect™ Cronobacter species PCR Assay method is certified for the detection of *Cronobacter* species within the scope of Tables 1 and 2.

Certification includes:

1. Applied Biosystems™ QuantStudio™ 5 Food Safety Real-Time PCR Instrument, 0.1-mL block, with RapidFinder Analysis Software v1.1 or later. For use with SureTect Cronobacter species PCR Assay and Pathogen Assay File: **Cronobacter spp-ST-A56845-QS5-2.2** or later — A36320 (desktop), A36328 (laptop).
2. Applied Biosystems™ 7500 Fast Real-Time PCR Instrument with RapidFinder Express Software v2.0 or later. For use with SureTect Cronobacter species PCR Assay and Pathogen Assay File: **Cronobacter_species_SureTect_7500_2.0** or later — A30304 (desktop), A30299 (laptop).
3. Applied Biosystems™ SimpliAmp™ Thermal Cycler

Table 1. Method Performance Claims

Matrix	Test portion	Enrichment Conditions				Reference Method ^b	Claim ^c
		Broth ^a	Volume	Time	Temperature		
Powdered infant formula with probiotics	10 g	BPW	90 mL	16-24 h	34–38°C	ISO 22964:2017	Eq
Powdered infant formula w/out probiotics	10 g	BPW	90 mL	16-24 h	34–38°C	ISO 22964:2017	Eq
Powdered infant formula with probiotics	375 g	pre-w BPW + 6 mg/L novobiocin	1,875 mL	18–26 h	34–38°C	ISO 22964:2017	NSDD
Powdered infant formula w/out probiotics	375 g	pre-w BPW	1,875 mL	18–26 h	34–38°C	ISO 22964:2017	NSDD
Stainless steel environmental surface	Swab, (1" x 1")	BPW	10 mL	20–28 h	34–38°C	ISO 22964:2017	NSDD
Process water	25 mL	BPW + 6 mg/L vancomycin	225 mL	18–26 h	34–38°C	ISO 22964:2017	NSDD

^a BPW = Buffered peptone water (ISO formulation); pre-w BPW + 6 mg/L novobiocin = pre-warmed BPW (ISO) with 6 mg/L novobiocin; pre-w BPW = pre-warmed BPW (ISO); BPW + 6 mg/L vancomycin = BPW (ISO) with 6 mg/L with 6 mg/L vancomycin.

^b EN ISO 22964:2017, Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

^c NSDD = No statistical difference detected using SLV study design from OMA Appendix J (2012). The SLV qualitative method comparison study design from OMA Appendix J (2012) is not intended to demonstrate statistical equivalence. Expert opinion is that the method is appropriate for its intended use. Eq = Equivalence of candidate and reference method results demonstrated by 90% confidence interval on dPOD_c meeting the criteria according to TR364.

Table 2. Method Selectivity in Ground Corn

Broth ^a	Enrichment	Temp., °C	Inclusivity Strains		Exclusivity Species	
			No. Tested	No. Positive	No. Tested	No. Positive
BPW with 6 mg/L novobiocin		34–38°C	56 ^b	56	30 ^c	0

^a BPW = Buffered peptone water (ISO formulation) with 6 mg/L novobiocin.

^b Comprised of 2 strain *C. condimenti*, 6 strains *C. dublinensis*, 3 strains *C. malonaticus*, 3 strains *C. muytjensii*, 29 strains *C. sakazakii*, 10 strains *C. turicensis*, and 3 strains *C. universalis*.

^c 30 strains comprising 30 species. Exclusivity organisms were cultured under optimal conditions for growth.

Table 3. Method History

No.	Date	Summary	Supporting Data
1	September 2024	Original Certification.	Certification Report (link pending)