Food testing

Validation of a rapid culture media workflow according to ISO 16140-2:2016 for the detection of *Cronobacter* species from selected matrices

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INTRODUCTION

Cronobacter species are ubiquitous organisms that are found in dried powders, including powdered infant formula (PIF). *Cronobacter* infections are particularly concerning for patients with suppressed immune systems, such as neonates, with case mortality reported to be between 50-80%.

The Thermo Scientific™ Cronobacter Precis™ workflow (alternative method) was evaluated according to EN ISO 16140-2:2016[1] against ISO 22694:2017[2] (reference method) for the detection of *Cronobacter* in PIF and environmental samples for MicroVal accreditation. The workflow offers a shorter time to result (<28 hours) than traditional methods.

The Cronobacter Precis Method is part of the Thermo Scientific™ Precis™ Method portfolio that also includes validated methods for *Salmonella* species, *Listeria* species and *L. monocytogenes*. After an overnight enrichment, a 10 µL loop is used to streak onto Thermo Scientific™ *Brilliance*™ chromogenic plates with negative and presumptive-positive results available in under 48 hours.

METHODS

The validation study evaluated PIF with and without probiotics using a paired study design for 10 g samples, and unpaired study design for up to 375 g and 25 g environmental samples together with surface sampling. Results are shown in figures 1 and 2.

Sensitivity Study

A total of 199 samples were tested according to 16140-2 guidelines.

Relative Limit of Detection (RLOD) Study

The RLOD study contained three categories, with three levels of contamination per category.

Inclusivity/Exclusivity

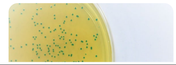
A total of 57 inclusivity and 31 exclusivity isolates were tested.

WORKFLOW

Enrichment

Thermo Scientific™ Oxoid™ Buffered Peptone Water (BPW) + 6 mg/L vancomycin for 375 g PIF, incubated at 34-38°C.

16-28hr



Confirmation

Culture using Thermo Scientific™ Oxoid™ Chromogenic Cronobacter Isolation Agar

Figure 1. Cronobacter Precis workflow

RESULTS

Figure 2. Sensitivity study results. PD = Positive Deviation, ND = Negative Deviation, NA = Negative Agreement, PA = Positive Agreement

Matrix	RLOD	Acceptability Limit (AL)
10 g PIF (with & without probiotics)	1.000	1.5
375 g PIF (with & without probiotics)	1.146	2.5
25 g Environmental/Surface Samples	1.157	2.5

Figure 2. RLOD study results

The difference between negative and positive deviations for each matrix category was assessed in the sensitivity study; all results were below the acceptability limit (AL) of 3, showing a comparable sensitivity to the reference method.

All categories tested were below the AL for the RLOD study, showing comparable limit of detection to the reference method.

Every inclusivity/exclusivity isolate tested gave the correct result.

CONCLUSIONS

Performance

- 100% specific and selective.
- Superior performance to ISO 22964:2017 – 92.3% compared to 86.8% respectively.

Time to Result

- Results within 28 hours, with only one enrichment needed.

MicroVal Certified

- Granted – **no. 2020LR93**

REFERENCES

1. ISO 16140-2:2016, Microbiology of the food chain - Method validation - Part 2:Protocol for the validation of alternative (proprietary) methods against a reference method (2016), ISO, https://www.iso.org/standard/54870.html (accessed March 2022)
2. ISO 22964:2017, Microbiology of the food chain – Horizontal method for the detection of Cronobacter spp., ISO, https://www.iso.org/standard/64708.html, (accessed March 2022)

TRADEMARKS/LICENSING