Food testing

ISO 16140-2:2016 extension study for a *Cronobacter* spp. PCR Assay to include up to 375 g powdered infant formula, infant cereals and related ingredient matrices

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INTRODUCTION

Cronobacter species are opportunistic pathogens predominantly found in dried powders, specifically powdered infant formula (PIF) and related ingredients. *Cronobacter* infections are of concern for patients with weakened immune systems, particularly neonates, with case mortality reported to be between 40-80%[1].

The Thermo Scientific™ SureTect™ Cronobacter PCR Assay method (alternative method) offers a rapid and easy-to-use workflow for detection of *Cronobacter* spp. and was evaluated according to the EN ISO 16140-2:2016[2] against ISO 22694:2017[3] to extend the scope of validation matrices to include up to 375 g PIF with and without probiotics, infant cereals and related ingredients. The workflow is outlined in figure 1.

Single enrichment → Rapid time to result of 26 hours → Easy chromogenic culture confirmation

METHODS

The validation extension study evaluated 375 g PIF, infant cereals and related ingredients (with and without probiotics) using an unpaired study design.

Sensitivity Study

A total of 66 samples were tested according to 16140-2 guidelines for the extension study.

Relative Limit of Detection (RLOD) Study

The RLOD study comprised of 30 samples across three contamination levels.

Inclusivity/Exclusivity

Data from original validation study was used comprising 57 inclusivity and 31 exclusivity isolates. Results showed 100% specificity.

WORKFLOW

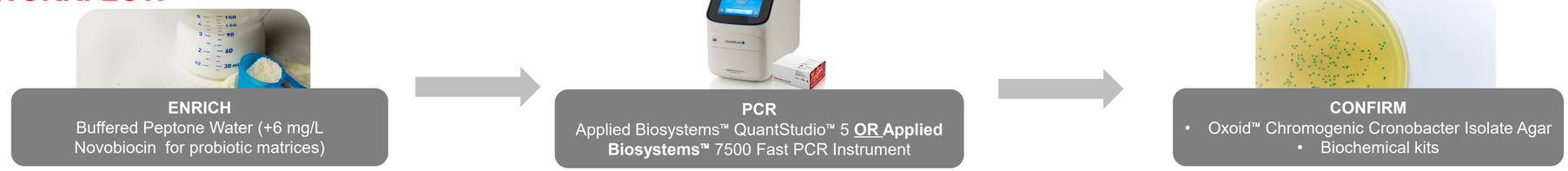

Figure 1. SureTect Cronobacter PCR assay workflow

RESULTS

For the 66 samples tested, the SureTect Cronobacter PCR Assay method was below the stipulated acceptability limit of 3. It also demonstrated higher sensitivity compared to the reference method.

Sensitivity	
SureTect Cronobacter Assay	ISO 22694:2017 Reference method
86.7%	76.7%

Figure 2. Up to 375 g PIF Sensitivity Study results. PD = Positive deviation, ND = Negative deviation, NA = Negative agreement, PA = Positive agreement

Matrix	RLOD	Acceptability Limit
PIF, Cereals and Related Ingredients	1.146	2.5

The RLOD was below the acceptability limit for the alternative method demonstrating a comparable limit of detection (LOD) to the reference method, showing the alternative method as a reliable alternative to the ISO 22964:2017 method.

Table 1. RLOD study results

CONCLUSIONS

Performance

- 100% specific and selective.
- Superior performance to ISO 22964:2017 with greater sensitivity.

Time to Result and Ease of Use

- Negative and presumptive-positive results in under 20 hours, with single enrichment.

NF Certified

- Granted – **no. UN03111215**

REFERENCES

1. Norberg *et al. Cronobacter* spp. in Powdered Infant Formula (2012) *Journal of Food Protection*, vol 75, issue 3: 607-620.
2. 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)
3. 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)

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