Performance Evaluation of a Real-time PCR for the Detection of *Cronobacter* spp. in Powdered Infant Formula.

VIKRANT DUTTA, PETER LADELL, NIKKI PALEN, JOHN C. MILLS, AND JOSEPH S. BAILEY
BIOMÉRIEUX, HAZELWOOD, MO

P3-83 ◦ IAFP 2018 - Salt Lake City, UT July 8-11, 2018

INTRODUCTION

Cronobacter spp. is a neonatal pathogen associated with dehydrated PIF. It can cause rare but life-threatening forms of neonatal meningitis, septicemia, necrotizing enterocolitis, and necrotizing meningo-encephalitis. The ubiquitous nature along with the resistance adaptive attributes of *Cronobacter* spp., like desiccation and osmotic stress resistance, makes it a persistent cause of concern for the powdered infant formula (PIF) manufacturing facilities. Therefore, despite all the mitigation steps employed at these facilities, testing the final product remains an effective strategy to detect *Cronobacter* contaminated powdered infant formula . The GENE-UP® *Cronobacter* spp. assay (CRO) is a Real-Time PCR assay that utilizes Fluorescence Resonance Energy Transfer (FRET) hybridization chemistry to provide highly sensitive multi-target detection in the same reaction vessel from a crude DNA preparation. The GENE-UP platform is highlighted by an easy to use workflow and overall flexibility.

PURPOSE

The goal of this study was to investigate the performance of CRO method for the detection of *Cronobacter spp.* in various types of PIF. The AOAC validation guidelines were used. The evaluation consisted of the following studies: inclusivity/exclusivity, method comparison of 4 food and 2 environmental matrices, lot-to-lot/stability and robustness. This poster presentation will focus specifically on the powdered infant formula method comparison portion of the validation study.

MATERIALS & METHODS

Two portion sizes (25g and 375g) of several types of powdered infant formula were evaluated in the study:

- **Milk-based Powdered Infant Formula with Probiotics,**
- **Milk-based Powdered Infant Formula without probiotics, and**
- **Soy-based powdered infant formula.**

Each product was inoculated with a different species of *Cronobacter* at two different levels (Table 1). For each product, five replicates were un-inoculated (0 CFU/test portion), twenty replicates were inoculated at a low level (0.2-2 CFU/test portion), and five replicates were inoculated at a high level (2-5 CFU/test portion). All test portions were enriched in buffered peptone water (BPW) and both the large sample sizes as well as products with probiotics require the addition of novobiocin (10mg/L). 375g samples were enriched at 1:6 using pre-warmed BPW and 25g samples were enriched at 1:10 with room temperature BPW. 25g test portions were incubated for 18h and 375g test portions were incubated for 20h, all at 37±1°C. Enrichments were analyzed by the CRO PCR method. All enrichments were biochemically confirmed by both the ISO22964 reference method and by an alternative method. The CRO method workflow for each portion size of the food products tested is shown in Figure 2. The data was analyzed as per the {dPOD} analysis.

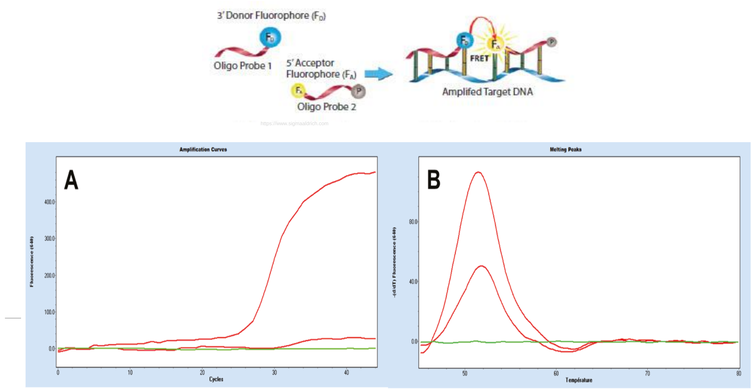

Figure 1*. FRET Real time PCR results of Salmonella spp.* ***A****. Amplification curves* ***B****. Melting curves*

Table 1. POD analysis for the detection of *Cronobacter* spp. in 375g and 25g powdered infant formula using GENE-UP® CRO PCR method versus the ISO22964 method.

Matrix/Test Portion	Strain	Time Point	Spike level	N[b]	Presumptive				Confirmed				dPOD$_C$[f]	95% CI[g]	
					x[c]	POD$_C$[d]	95% CI		x[c]	POD$_R$[e]	95% CI				
Milk-based powdered infant formula with probiotics (25g)	*Cronobacter sakazakii* ATCC 29544	18h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	13	0.65	0.43	0.82	13	0.65	0.43	0.82	0.00	-0.28	0.28
			High	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (25g)	*Cronobacter dublinensis spp dublinensis* BMX 112161	18h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	14	0.70	0.48	0.85	14	0.70	0.48	0.85	0.00	-0.27	0.27
			High	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (25g)	*Cronobacter sakazakii* ATCC BAA-894	18h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	7	0.35	0.18	0.57	7	0.35	0.18	0.57	0.00	-0.28	0.28
			High	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula with probiotics (375g)	*Cronobacter sakazakii* ATCC 29544	20h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	17	0.85	0.64	0.95	17	0.85	0.64	0.95	0.00	-0.23	0.23
			High	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Milk-based powdered infant formula without probiotics (375g)	*Cronobacter dublinensis spp dublinensis* BMX 112161	20h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	16	0.80	0.58	0.92	16	0.80	0.58	0.92	0.00	-0.25	0.25
			High	5	5	1.00	0.57	1.00	5	1.00	0.57	1.00	0.00	-0.43	0.43
Soy-based powdered infant formula (375g)	*Cronobacter sakazakii* ATCC BAA-894	20h	Uninoculated	5	0	0.00	0.00	0.43	0	0.00	0.00	0.43	0.00	-0.43	0.43
			Low	20	9	0.45	0.26	0.66	9	0.45	0.26	0.66	0.00	-0.28	0.28
			High	5	4	0.80	0.38	1.00	4	0.80	0.38	1.00	0.00	-0.47	0.47

[b]N = Number of test portions
[c]x = Number of positive test portions
[d]POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials
[e]POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials
[f]dPOD$_C$= Difference between the confirmed candidate method result and reference method confirmed result POD values
[g]95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level
[h]CFU/Test Area = Results of the CFU/Test area were determined by plating the inoculum for each matrix in triplicate

Figure 2. GENE-UP *Cronobacter* spp: General protocol powdered infant formula.

STUDY HIGHLIGHTS

- No statistically significant differences were observed between the performance of GENE-UP CRO and the ISO22964 reference method (dPOD: 0.0 with 95% confidence interval).
- Equivalent performance was observed for raw material matrices i.e. soy protein, non-fat dry milk, and the environmental surfaces i.e. stainless steel, and plastic (data not shown here).
- These data support the high sensitivity and use of GENE-UP CRO for the detection of *Cronobacter spp.* in 25g and 375g powdered infant formula with and without probiotics.
- The CRO workflow provides a ~24h time advantage to results over the traditional culture method.

ACKNOWLEDGEMENTS

We would like to thank our colleagues at BIOMÉRIEUX, Hazelwood (U.S.), Grenoble and Marcy-l'Étoile (France), and to our associates at BioFire Defense, Inc. (Salt Lake City, UT) for all their support and encouragement.

References:

•ISO TS 22964:2017 – Horizontal method for the detection of *Cronobacter* spp.
•AOAC Research Institute Performance Tested Methods℠ protocols listed in the Official Methods of Analysis of AOAC INTERNATIONAL