

Evaluation Of The Thermo Scientific SureTect Real-Time PCR Assay Method For Detection Of *Cronobacter* Species In Powdered Infant Formula

Helen Rose¹, Katharine Evans¹, David Crabtree¹, Yee-Ling Gregg¹, Gavin Bingley¹, James Stringer¹, Jani Holopainen², Mikko Kauppinen²

¹Thermo Fisher Scientific, Basingstoke, Hampshire, UK and ²Thermo Fisher Scientific, Vantaa, Uusimaa, Finland

Overview

Purpose: To demonstrate the performance of Buffered Peptone Water (BPW) supplemented with 6 mg/l vancomycin as a suitable enrichment medium for the detection of *Cronobacter* species in 300 g samples of probiotic and non-probiotic powdered infant formula (PIF) when using the Thermo Scientific™ SureTect™ *Cronobacter* species PCR Assay (alternative method).

Methods: The alternative method was compared to the draft ISO reference method as detailed in ISO22964:DIS2015.

Results: The alternative method correctly identified an additional 11% of probiotic PIF samples as positive compared to the draft ISO reference method after 16 hours enrichment, rising to 33% after 19 hours. The alternative method was comparable to the draft ISO reference method for non-probiotic PIF.

Introduction

Draft ISO22964:DIS2015 prepares samples by adding x g of test sample to 9x ml BPW which provides sample size flexibility. European Union Commission Regulation (EC) No 2073/2005 states that dried infant formula placed on the market within the EU must be analysed for *Cronobacter* spp as 30 samples of 10 g. Draft ISO22964:DIS2015 allows these aliquots to be pooled into a single 300 g sample. Testing fewer samples streamlines laboratory workflows, increases testing capacity and may provide benefits of reduced costs through the use of reduced consumables and fewer handling steps. The SureTect *Cronobacter* species Assay has previously been validated according to ISO16140 in the context of NF VALIDATION™ by AFNOR Certification for 10 g PIF (with and without probiotics) and production environment samples. PIF is supplemented with probiotics to prevent acute infectious diarrhoea in infants¹ and is available in Europe, Asia and North America². The high levels of lactic acid bacteria found in probiotic PIF reduce the pH of the enrichment during incubation, causing inhibition of *Cronobacter* growth. When PIF is contaminated with very low numbers of *Cronobacter*, larger samples will have a greater ratio of lactic acid bacteria to *Cronobacter* compared to smaller sample sizes. This could further amplify the pH drop and inhibit growth of *Cronobacter* spp.³.

Methods

Sample Preparation

A total of 29 samples from five probiotic and three non-probiotic PIF brands were spiked with 3-10 CFU/sample of stressed *Cronobacter* isolates.

Alternative Method

Three hundred gram PIF samples were diluted in 2.7 litres BPW + 6 mg/l vancomycin and incubated at 37±2°C for 16-19 hours. Post incubation, enrichments were tested at 16 and 19 hours using the SureTect PCR Assay and the pH was analysed. Confirmations as detailed in draft ISO22964:DIS2015 were performed for all enrichments by sub-culturing 100 µl of enrichment into 10 ml Thermo Scientific™ Oxoid™ *Cronobacter* Screening Broth, incubated at 41.5±1°C for 24±2 hours, followed by inoculation onto Thermo Scientific™ Oxoid™ Chromogenic *Cronobacter* Isolation Agar, incubated at 41.5±1°C for 24±2 hours.

Draft ISO Reference Method

Ten gram PIF samples were diluted in 90 ml BPW and incubated at 37±2°C for 16-19 hours. Confirmations as detailed in draft ISO22964:DIS2015 were performed for 16 and 19 hour enrichments by sub-culturing 100 µl of enrichment into 10 ml *Cronobacter* Screening Broth, incubated at 41.5±1°C for 24±2 hours, followed by inoculation onto Chromogenic *Cronobacter* Isolation Agar, incubated at 41.5±1°C for 24±2 hours. The pH was assessed after 16 and 19 hours enrichment.

Results

As tables 1-3 show, there were frequent positive deviations when probiotic PIF was enriched following the alternative method compared to the draft ISO reference method. Both methods were comparable for non-probiotic PIF. The pH of the reference samples post enrichment were on average 2.0 pH units lower than the corresponding alternative method samples. Graph 1 shows the pH decrease over time of one probiotic PIF brand spiked with *C. sakazakii*. The decreased pH of the draft ISO reference samples was shown to cause growth inhibition or die off of *Cronobacter* over time, leading to false negative results.

TABLE 1. Study results for PIF (probiotic): Alternative Method Versus Draft ISO Reference Method

Incubation time (hours)	PA	NA	ND	PD	Total
16	9	5	1	3	18
19	7	5	0	6	18

TABLE 2. Study results for PIF (non-probiotic): Alternative Method Versus Draft ISO Reference Method

Incubation time (hours)	PA	NA	ND	PD	Total
16	7	3	0	1	11
19	7	3	0	1	11

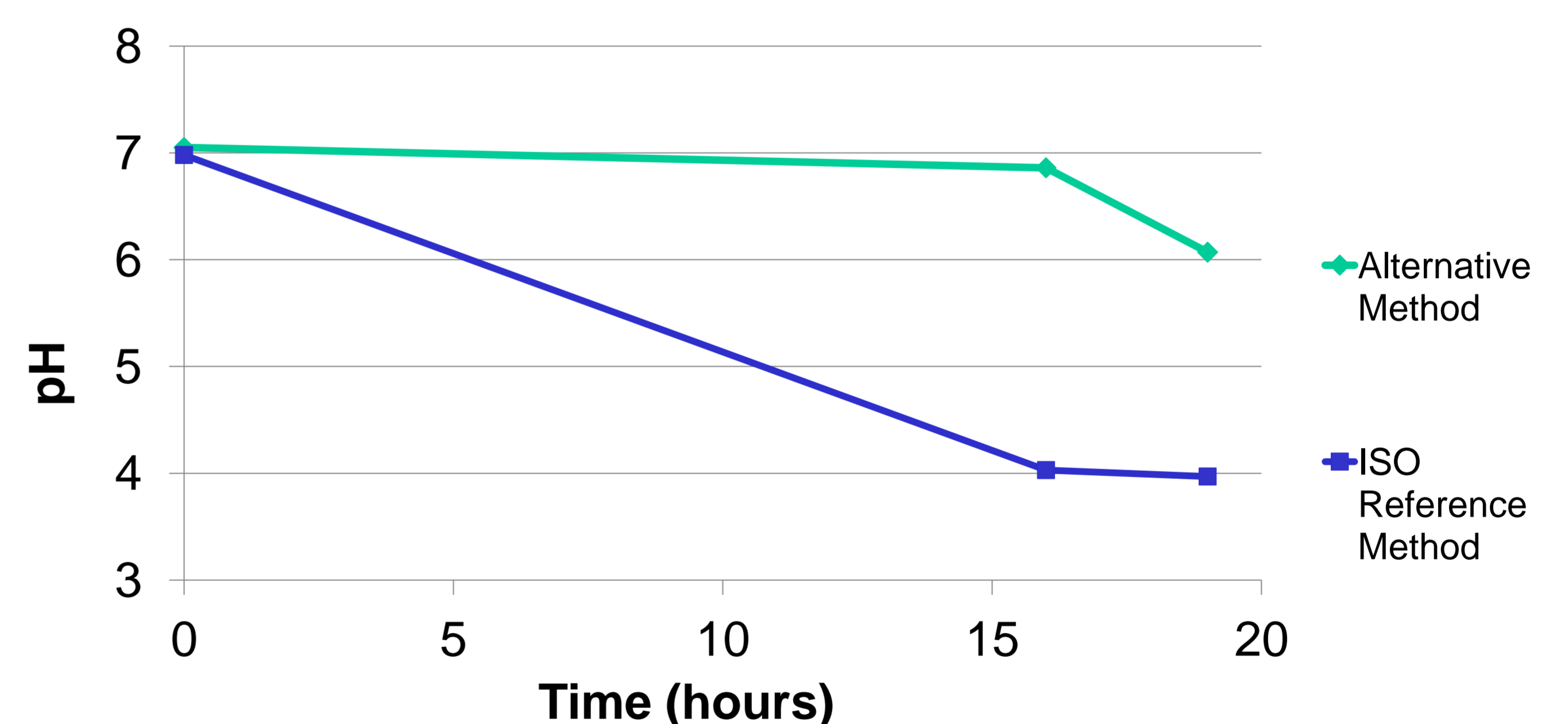
TABLE 3. Study results for PIF (probiotic and non-probiotic): Alternative Method Versus Draft ISO Reference Method

Incubation time (hours)	PA	NA	ND	PD	Total
16	16	8	1	4	29
19	14	8	0	7	29

Key

- PA - Positive agreement (both methods positive)
- NA - Negative agreement (both methods negative)
- ND - Negative deviation (PCR negative, ISO positive)
- PD - Positive deviation (PCR positive, ISO negative)

GRAPH 1. Example of the pH drop of PIF Enrichments After 0, 16 and 19 Hours Incubation



Conclusion

The performance of the alternative method with 300 g PIF samples outperformed the analysis of 10 g samples with the draft ISO reference method. Supplementation of the enrichment broth with vancomycin was necessary to recover low levels of *Cronobacter* present in probiotic PIF, which were missed by the draft ISO method.

References

1. Thomas D., Greer F. (2010). Probiotics and Prebiotics in Pediatrics. American Academy of Pediatrics. 126:1217-1231
2. Collier R. (2009). Squabble over risks of probiotic infant formula. CMAJ. 181(3-4):E46-E47
3. Miled R. B., Neves S., Baudouin N., Lombard B., Deperrois V., Colin P., Besse N. G. (2010). Impact of pooling powdered infant formula samples on bacterial evolution and *Cronobacter* detection. International Journal of Food Microbiology. 138:250-259