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APPLICATION NOTE

Detection of Cronobacter from Non-Probiotic Powdered Infant Formula and Milk Powder

Authors: Annette Hughes, Katharine Evans, David Crabtree
Thermo Fisher Scientific,
Wade Road, Basingstoke, Hampshire,
United Kingdom, RG24 8PW

Introduction

NF VALIDATION™ certification by AFNOR has been granted for the use of the Thermo Scientific™ SureTect™ Cronobacter PCR Assay for the detection of *Cronobacter* species from 300 g powdered infant formula (PIF) samples using the Applied Biosystems™ 7500 Fast Real-Time PCR Instruments (refer to Figure 1). The validated enrichment method uses 2.7 liters of BPW, with 6 mg/L of vancomycin incubated at 37±1°C for 16–20 hours.

The requirement for a vancomycin addition with PIF and milk powders is to inhibit growth of probiotic organisms, such as *Bifidobacterium* and *Lactobacillus*, if they are included in the product. These probiotic organisms lower the pH of the enrichment during incubation causing the *Cronobacter* to die off over time. This may allow DNA from non-viable cells to cause positive PCR results that cannot be confirmed through culture methods.

This study was conducted to evaluate performance of BPW without vancomycin as the enrichment medium for 300 g samples of PIF and other milk powders which do not contain probiotic organisms vs. the reference method, ISO 22964:2017.

Figure 1. Applied Biosystems 7500 Fast Food Safety Real-Time PCR System and Thermo Scientific SureTect Cronobacter species PCR Kit.

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Materials and methods

Twenty-seven PIF samples and three milk powder products were tested using the process flows shown in Figures 2 and 3.

Samples were spiked with low levels of *Cronobacter* species that had been injured by desiccation.

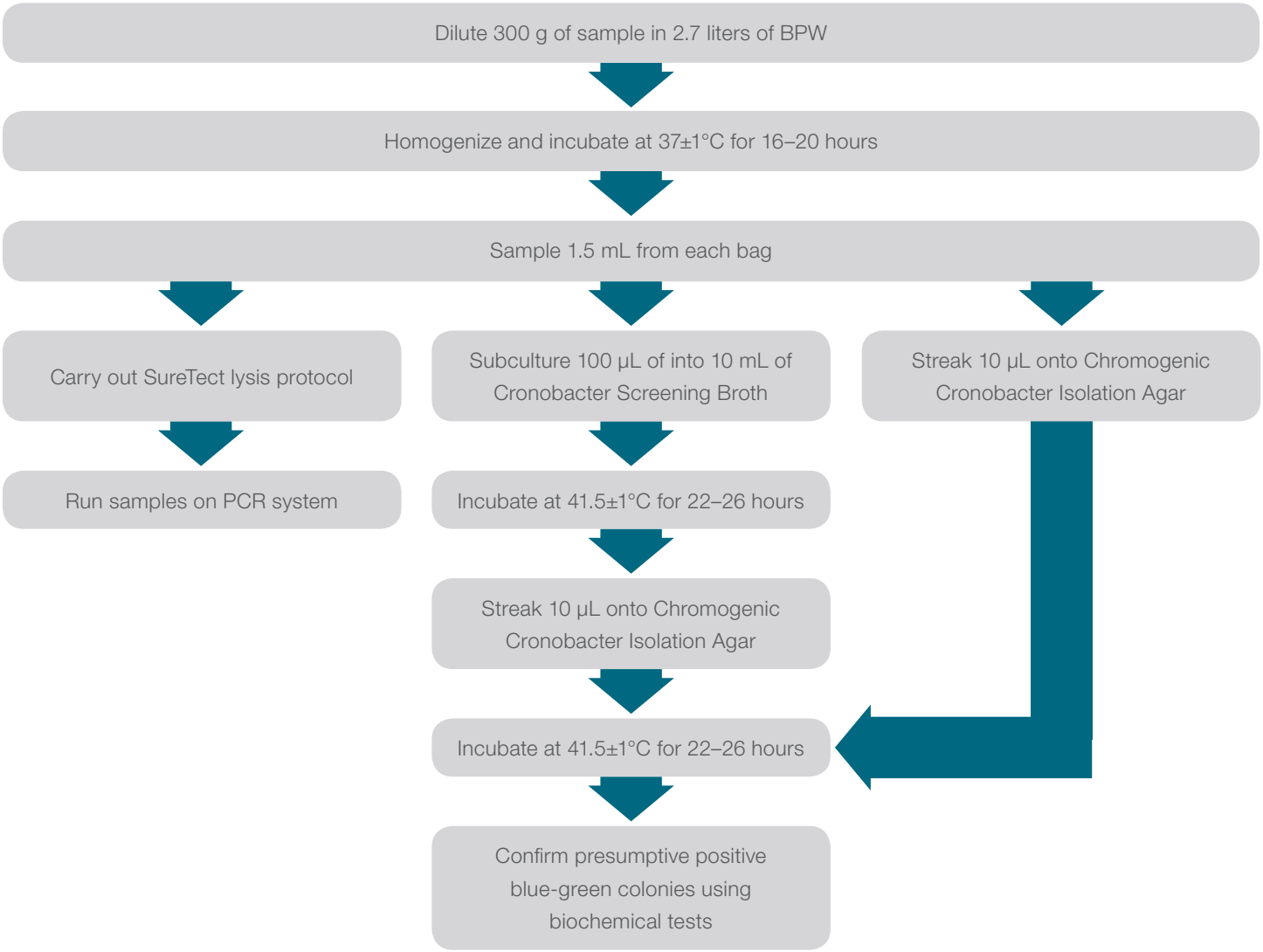

Figure 2. Process flow for the SureTect Cronobacter PCR Assay method for PIF and milk powder.

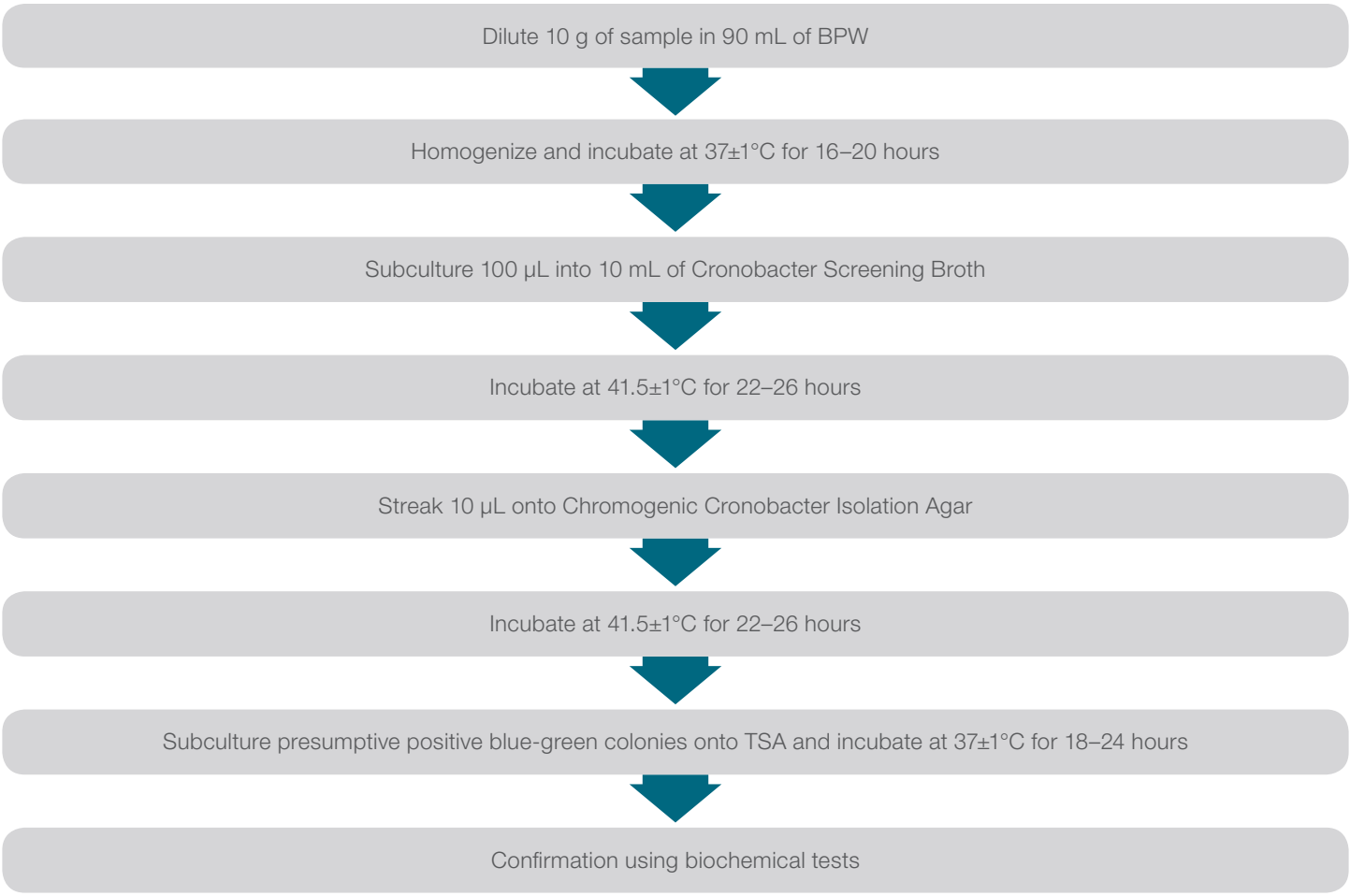

Figure 3. Process flow of ISO 22964:2017 for PIF and milk powder.

Results

Table 1. SureTect PCR assay results for PIF vs ISO 22964:2017 after 16 hours incubation.

Matrix	Number of replicates	PA	NA	ND	PD
PIF	27	17	9	0	1

Table 2. SureTect PCR assay results for other milk powder products vs ISO 22964:2017 after 18 hours incubation.

Matrix	Number of replicates	PA	NA	ND	PD
Skimmed milk powder (0.5% fat)	8	5	3	0	0
Whole milk powder (26% fat)	8	5	2	1	0
Cream milk powder (50% fat)	8	5	3	0	0

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)

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The performance of the SureTect Cronobacter PCR Assay was comparable to the ISO reference method for detecting *Cronobacter* species from non-probiotic PIF samples when vancomycin was not added to the primary enrichment broth (Table 1).

For the other milk powders, 1 negative deviation was seen when using the SureTect Cronobacter PCR Assay (Table 2). This was likely to be caused by a low spike level in combination with the larger sample size.

The rest of the results were comparable to the ISO 22964:2017 reference method.

Conclusions

The results show that performance of the SureTect Cronobacter PCR Assay method was comparable to the reference method, eliminating the need for addition of vancomycin to the enrichment medium for the detection of *Cronobacter* from PIF and milk powders that do not contain probiotic organisms.

References

1. ISO 22964:2017 Horizontal method for the detection of *Cronobacter* spp.

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Contact Information:
microbiology@thermofisher.com
USA +1 800 255 6730
International +44 (0) 1256 84114

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