Methods for Detection of *Cronobacter* spp. in Food and Environmental Samples

Tech Note

Introduction

Cronobacter spp., formerly *Enterobacter sakazakii*, is a Gram-negative, motile, rod-shaped, non-sporulating pathogenic bacterium that can cause foodborne illness, primarily among infants and immunocompromised adults. It can cause rare but severe neonatal meningitis, bacteremia, and necrotizing enterocolitis. The organism is able to survive in low moisture foods, such as powdered infant formula, for long periods.

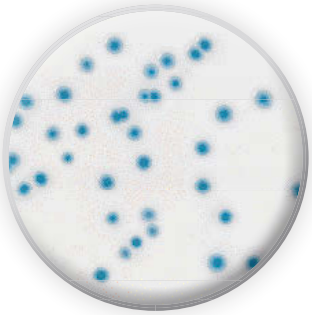
Cronobacter are ubiquitous and have been found in vegetables, fruit, spices, cereal, meat, powdered infant formula, rehydrated infant formula, utensils used to prepare infant formula, and other dairy products. The organism's high tolerance to desiccation provides it a competitive advantage in dry environments. *Cronobacter* spp. can survive in powdered infant formula for up to two years. Because *Cronobacter* cannot survive the pasteurization process, contamination usually occurs after the drying process either through introduction from the manufacturing environment or through the addition of other ingredients.

Methodology

For years, cultural based methods such as ISO 22964:2006 and the FDA detection method have been used to screen food and environmental samples. These methods are time consuming and sometimes offered poor selectivity for *Cronobacter* in the presence of competing flora. Following the ISO 22964:2006 method, presumptive results can be determined after 3–5 days and positive results in 6 days. Using the FDA culture method, confirmed results can be from 3–5 days. Recently, the FDA has completed the validation of a new method for the detection of *Cronobacter* in powdered infant formula using real-time PCR technology. Real-time PCR achieves faster time to results and increased selectivity. Bio-Rad has added to its extensive line of real-time PCR assays a *Cronobacter* detection method that uses a single enrichment that yields next day results for product and environmental samples.

The iQ-Check™ *Cronobacter* spp. is a test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain DNA primers and a DNA probe specific for *Cronobacter* spp., as well as DNA polymerase and nucleotides. Detection and data analysis are optimized for use with Bio-Rad real-time PCR instrumentation and data analysis software. The genetic target of Bio-Rad's iQ-Check *Cronobacter* spp. Kit is similar to the FDA's real-time PCR method.

Environmental samples are enriched in buffered peptone water for 18 hours ± 2 hours at 37°C ± 1°C. Following incubation, DNA extraction of samples occurs. Finished product is enriched in buffered peptone water supplemented with vancomycin for 20 hours ± 2 hours at 37°C ± 1°C. After the first enrichment, samples are transferred to a secondary enrichment in buffered peptone water for 4 hours ± 2 hour at 37°C ± 1°C. DNA extraction of sample occurs following this secondary enrichment. Presumptive product can be confirmed by direct streaking of the enrichment onto RAPID'*Sakazakii* chromogenic agar. Presumptive environmental samples can be confirmed on RAPID'*Sakazakii* chromogenic agar after 24 hour enrichment in mLST.



RAPID'*Sakazakii* Agar

Bio-Rad's iQ-Check *Cronobacter* spp. Kit used in conjunction with RAPID'*Sakazakii* chromogenic agar gives fast, accurate results with increased selectivity and sensitivity, ensuring the quality of your results. The iQ-Check *Cronobacter* spp. kit has received NordVal certification and NF (AFNOR) validation (Table 1). The reference method used for validation is ISO/TS 22964 (2006) — Milk and dairy products — Horizontal method for the detection of *Enterobacter sakazakii.*

For more information regarding our *Cronobacter* testing solutions or to learn about all of our food testing products, visit our website at **www.foodscience.biorad.com.**

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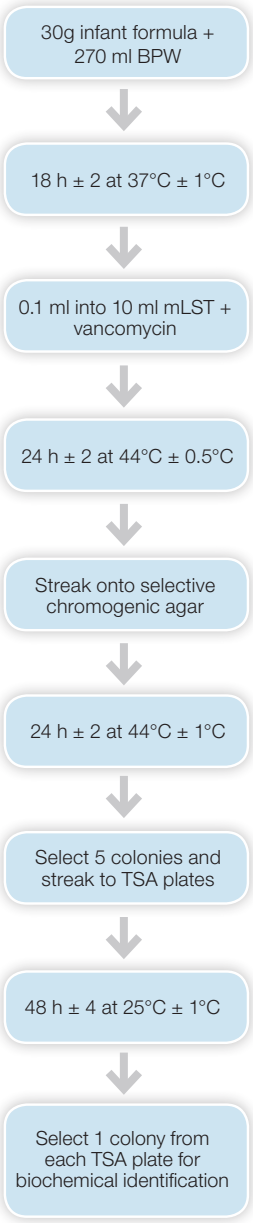
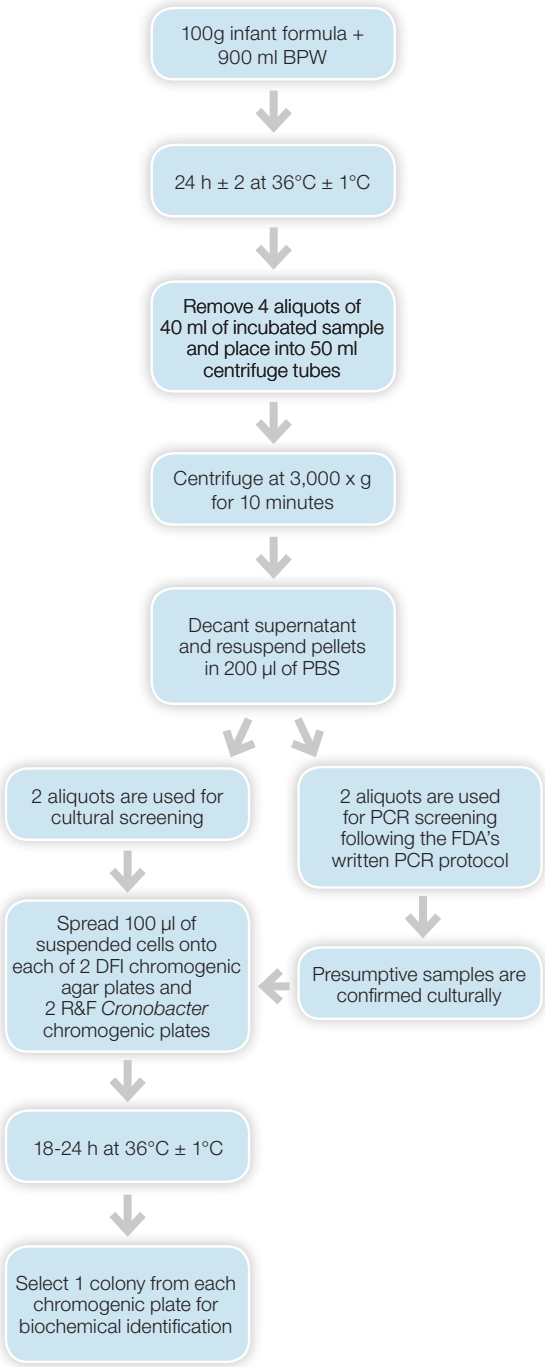
Table 1. Validation Results for Product and Environmental Samples

Relative Accuracy	Relative Sensitivity	Relative Specificity	Limit of Detection	Inclusivity	Exclusivity
97%	99%	98%	1-10 cfu per 30 g/mL	52/52 Strains	31/31 Strains

ISO/TS 22964:2006

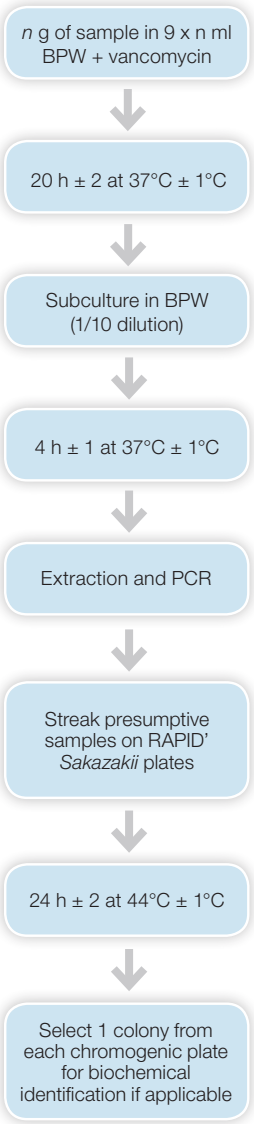


FDA BAM Chapter 29



iQ-Check *Cronobacter* spp. Method

Dairy Samples



Environmental Samples