Cronobacter: A Two Part Series

Part I: The *Cronobacter* Impact

Cronobacter species are gram-negative, facultative, anaerobic rod-shaped bacteria, and part of the Enterobacteriaceae family. Previously described as a single species named *E.sakazakii*, it has been reclassified in the *Cronobacter* genus, currently comprising 7 species, all potentially pathogenic. Naturally occurring in the environment, *Cronobacter* can survive extremely dry conditions and other stress mechanisms. Combined, these resistance attributes make *Cronobacter* a persistent problem for milk powder, infant formula, and milk- and plant-based dry manufacturing facilities.

Many consumers associate *Cronobacter* spp. primarily with contaminated neonatal and powdered infant formulas (PIF)—and the resulting clinical manifestations like septicemia, meningitis, and necrotizing enterocolitis. While the majority of the attention to *Cronobacter* is understandably focused on the premature and immune-compromised newborns who are the primary victims of these rare bacterial infections, *Cronobacter* has also been linked to clinical cases of infection in older infants, as well as adult bacteremia.[1]

Severe *Cronobacter* infections are most always linked to infant formula—and despite implementing risk-reduction best practices such as validated process controls, environmental monitoring programs, and supplier verification programs, PIF manufacturers still rely on proactive testing of both raw materials and finished goods. Although less reported, *Cronobacter* has also been implicated with other raw materials like nonfat dry milk and whey, dry foods like herbal teas and starches, and in-process water.

In order to implement effective controls along the food supply chain and at home—and reduce the number of *Cronobacter* spp. infections in infants—a multidisciplinary approach involving producers, processors, retailers, and the consumer is necessary. According to the Centers for Disease Control and Prevention (CDC), some cases of

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Cronobacter contamination might have occurred after the powdered infant formula was opened or prepared at home.[1]

With guidelines in place like the Annex I to Regulation (EC) No 2073/2005, EU and U.S. regulatory agencies and dairy companies are taking action as part of an international effort to make raw materials, milk powders, powdered infant formulas, and surface both safer and pathogen free.

References:
1 Retrieved from https://www.cdc.gov/cronobacter/technical.html

Part II: *Cronobacter* Testing Made Simple

Reports of *Cronobacter* infections in humans have mostly been linked to contaminated powdered infant formula (PIF). Current international microbiological regulations require complete eradication of *Cronobacter* species from PIF, assuring food safety for consumers that are both sensitive and vulnerable. To better protect public health, specific, sensitive, and rapid detection and identification methods have become highly sought after. In addition to preventive efforts, PIF manufacturers also rely on the microbial test and ID systems to control *Cronobacter* in their products and environment. When it comes to *Cronobacter* detection, few issues arise when dealing with clinical samples; unfortunately, this isn't the case when it comes to industrial samples. The uneven distribution, low prevalence and stressed state of *Cronobacter*—as well as the complexity of various matrices—makes the quest to detect this pathogen a cumbersome experience.

When testing raw materials, finished goods, and environmental surfaces for Cronobacter, rapidity and convenience are of the essence, as pre-enrichment is required before testing in order to resuscitate cells to detectable levels for the selected method. Cultural procedures require: pre-enrichment, and selective enrichment, followed by isolation on chromogenic media. These cultural methods are most often used and followed by regulatory agencies (e.g. FDA-BAM Chapter 29, ISO 22964:2017). Also, specific short chromogenic protocols can be used immediately after a unique selective enrichment while remaining a cultural technique. On the other hand, molecular methods can be used immediately after pre-enrichment with no subsequent selective enrichment. It is also possible to target and concentrate *Cronobacter* cells after enrichment to help increase the number of cells, but this process can introduce more touch points and potential risk for contamination.

As per the FDA-BAM (Bacteriological Analytical Manual) Chapter 291, the standard method includes a PCR method for screening detection, followed by an isolation on chromogenic agars for confirmation. Chromogenic agars can also be used as a screening and isolation method, where presumptive positive results should always be confirmed biochemically (or via PCR) to confirm pure cultures of *Cronobacter* spp. On the other hand, ISO 22964:2017 only relies on the culture methods for screening and confirmation of Cronobacter spp.

Cultural methods are cumbersome and time consuming, and introduce challenges such as sample handling and transfers, multiple media manipulations, and expert interpretation of colonies. This is why a simple and convenient protocol, suitable for various sample sizes and matrix diversity—and with easy handling and interpretation—is key for implementation in the laboratory.

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Traditionally, a standard 10-gram sample size is prescribed by the FDA-BAM and EN ISO 22964:20172. However, the variety and complexity of samples and associated risk analysis—and the specific requirements of different industries—have led to deviations from the standard 10-gram sample, referenced in FDA's regulations published in Title 21 of the Code of Federal Regulations (CFR) 106.5523, or Annex I to Regulation (EC) No 2073/20054. Sampling plans based on risk analysis and management can require sample sizes varying from 100 g for gums and starches, to 375 g for soy-based powdered infant formula with probiotic supplements. Adding to this complexity is the industry desire for compositing or pooling for Cronobacter spp. and other Enterobacteriaceae testing, like Salmonella. Equally, crucial is defining a proper sampling plan along with an effective environmental monitoring program designed to eliminate Cronobacter spp. persistence in the PIF manufacturing environment, especially on the equipment surfaces and seemingly trivial crevices. One more complex facet to this testing is the increasing need for simultaneous testing for Salmonella and Cronobacter from the same enrichment.

Rapid and reliable detection methods for Cronobacter spp. are needed to alleviate workflow bottlenecks in the laboratory, provide data traceability, meet growing needs and challenges, while allowing earlier corrective actions at the production level. Partnering with diagnostic test providers can help PIF manufacturers address any deviation from standard methods like FDA, BAM, and ISO, and proactively focus on adjusting to their organization's unique needs.

While increased productivity and optimized workflow are vital to any manufacturer, an accurate and sensitive detection method for Cronobacter is critical for superior operational efficiency—not only for reducing unnecessary confirmation tests, investigations, and product quarantines, but mainly for ensuring consumer safety. In the era of pervasive social media, where a tweet or post can go viral in the blink of an eye, maximizing both operational efficiency and consumer safety have to be prioritized simultaneously. This is critical to protect both the brand reputation of manufacturers, and the Consumer Packaged Goods (CPGs) they produce.

References:
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