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# State-of-the-art *Cronobacter* confirmation

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MALDI-TOF MS is an excellent tool for confirming bacterial colonies thanks to its speed, accuracy, and cost-effectiveness, especially for *Cronobacter spp.* in powdered infant formula or its ingredients. Before the advent of MALDI-TOF MS, traditional biochemical assays, such as sugar fermentation tests and enzyme activity assays, were the cornerstone of bacterial identification. While these tests are simple and inexpensive, they suffer from a long time-to-result. Whereas the MALDI Biotyper® can be complemented by the IR Biotyper® for the possibility of isolate discrimination and same day root cause analysis via FT-IR.

*Cronobacter* spp. have been isolated from a wide range of sources, including various food products - especially dry and dried items - as well as surfaces in healthcare settings, food production facilities and private households. These bacteria can thrive in dry foods such as powdered infant formula and powdered milk and are also found in herbal teas, and starches.[1]

Powdered infant formula (PIF) is a meticulously manufactured alternative to breast milk, serving as a vital source of nutrition for infants worldwide. While it provides essential nourishment, infants and young children are particularly vulnerable to foodborne illnesses due to their still developing immune systems, highlighting the critical importance of ensuring PIF safety.[2]

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have extensively reviewed microbial contamination and toxins in PIF. Among the various harmful microorganisms identified, *Cronobacter* and *Salmonella* showed significant risks, having been conclusively linked to severe illnesses in infants, including developmental complications and fatalities. Therefore, monitoring and mitigating the presence of *Cronobacter* in PIF is crucial for safeguarding infant health.

According to the German Federal Institute for Risk Assessment (BfR) in Berlin, infections caused by *Cronobacter* spp. are exceedingly rare and primarily affect infants with a birth weight of less than 2,500 grams, as well as those with compromised immune systems.[3] Despite their rarity, these infections can lead to severe health consequences for affected infants. European food safety regulation (EC) No. 2073/2005 outlines strict microbiological criteria for infant formula, requiring the absence of *Salmonella* and *Cronobacter* spp. based on defined sampling plans. Additionally, the detection of other microorganisms, such as bacteria from the *Enterobacteriaceae* family or *Bacillus cereus*, helps to verify a hygienic manufacturing process.

It's important to also consider household safety. The WHO recommends using water with at least 70 °C for reconstitution, which, according to a mathematical model, leads to a four-log reduction in germs. However, in practice, the 70 °C water often cools too quickly to effectively eliminate *Cronobacter* bacteria.

In many food safety laboratories, the MALDI Biotyper solution is the gold standard for rapid confirmation of *Cronobacter* spp. and *Salmonella* spp. from microbial cultures. In 2018, MicroVal approved four validation studies against ISO 16140-6. These studies resulted in certificates for the MALDI Biotyper for four different target organisms (*Cronobacter* spp., *Salmonella* spp., *Campylobacter*, and *Listeria* spp. & *L. monocytogenes*).[4] Additionally, the MALDI Biotyper system was approved according to AOAC-OMA by AOAC INTERNATIONAL.

The MALDI Biotyper is an excellent tool for *Cronobacter* spp. confirmation. In a single-lab study, Stephan et al. (2020) developed a customized reference library that includes 54 *Cronobacter* target strains and 17 non-target strains.[5] This study demonstrated that MALDI-TOF MS is a reliable and powerful tool for the rapid identification of *Cronobacter* strains to the genus and species level in a single lab. Effective hygiene control and source tracking throughout food production lines require state-of-the-art monitoring technologies, which Bruker is dedicated to provide.

To date, only *C. sakazakii* (formerly *Enterobacter sakazakii*), *C. malonaticus* and *C. turicensis* have been associated with human diseases, with contamination of infant formula mainly due to *C. sakazakii* and *C. malonaticus*. The sequence type (ST) *C. sakazakii* ST4 has been identified as predominant in neonatal infections, but ST 8 and ST 12 are also considered pathogenic.[3]

The differentiation of *C. sakazakii* from other *Cronobacter* species is now achievable using lipid analysis in negative ion mode with MALDI-TOF MS in RUO mode. This pathogen can be reliably identified based on distinct Lipid A derivative ratio differences. At ESCMID Global 2025 (Poster P1990), Bruker's expert R&D team demonstrated that these peak ratios remain consistent regardless of the cultivation medium or incubation temperature. With the MALDI Biotyper sirius and its negative ion mode, species-level identification directly from colonies can now be completed in under an hour.

Bruker's latest industrial solution, the IR Biotyper, utilizes Fourier-transform infrared (FT-IR) spectroscopy to offer a novel approach for fast strain typing, providing results within just 3 hours. Designed as a screening tool, the IR Biotyper quickly identifies bacterial isolate relationships for root cause analysis.

With its high discriminatory power and a fast and easy workflow, the IR Biotyper allows species and strain monitoring, facilitating proper source-tracking.[6] Bruker offers customized species identification and strain typing of the relevant *Cronobacter* strains, achievable in less than 3 hours. The IR Biotyper serves as an early screening method/warning system, which can then be complemented by methods such as NGS/WGS.


References

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