Food safety

# Harmonized *Salmonella* and *Cronobacter* Testing in Powdered Infant Formula and Related Matrices

Powdered infant formula (PIF) is a widely used and essential nutritional source for infants who are unable to consume breast milk or do not have access to it. It is crucial to ensure the safety and quality of these products, as infants have developing immune systems that make them more susceptible to infection[1]. *Salmonella* and *Cronobacter* are two significant bacterial pathogens that pose potential health risks to infants, making the testing of PIF for these pathogens of utmost importance[2,3].

To ensure the safety of PIF and related matrices to consumers, Thermo Fisher Scientific offers comprehensive end-to-end workflows for the detection of *Salmonella* species and *Cronobacter* species. These workflows are specifically designed for testing PIF and related matrices, including environmental surfaces, and follow a harmonized sample preparation and enrichment protocol. The unique advantage of these workflows is that the same test portion can be used for both, providing a cost-effective and efficient testing solution.

The end-to-end workflows incorporate the highly reliable Thermo Scientific™ SureTect™ Salmonella species PCR Assay and Thermo Scientific™ SureTect™ Cronobacter species PCR Assay. These assays have been developed to simplify the testing process while ensuring the highest level of performance and fast time to actionable results. The PCR assays are known for their long-standing track record of sensitivity and specificity, enabling accurate detection of *Salmonella* and *Cronobacter* species in PIF and infant cereals and ingredients.

One of the key strengths of these workflows is their user-friendly design, which allows for streamlined testing processes. The protocols are straightforward and easy to follow, minimizing the potential for errors and ensuring consistent and reliable results. By providing a simplified and efficient testing solution, the worlkflow empowers laboratories to enhance their productivity and meet the demands of testing PIF and related matrices more effectively. The harmonized workflows for *Salmonella* and *Cronobacter* testing in PIF and environmental surfaces can be seen in Table 1.

**Table 1. Harmonized protocols for up to 375 g PIF and stainless-steel surface.**

| Matrices | Enrichment protocols | |
|---|---|---|
| | SureTect Salmonella PCR Assay | SureTect Cronobacter PCR Assay |
| Powdered Infant Formula with probiotics (up to 375 g) | 1-in-6 (1,875 mL) pre-warmed BPW + 6 mg/L novobiocin, 18–26 h at 34–38 °C | |
| Powdered Infant Formula without probiotics (up to 375 g) | 1-in-6 (1,875 mL) pre-warmed BPW, 18–26 h at 34–38 °C | |
| Stainless steel (1”x 1”; swab)[a] | 10 mL BPW, 20–28 h at 34–38 °C | 10 mL BPW, 18–26 h at 34–38 °C |

a. SureTect Cronoacter PCR Assay has a lower minimum enrichment time (2 h).

## Methods of verification

The performance of the PCR assays was verified through matrix studies which followed the guidelines outlined in *Official Methods of Analysis*℠ Appendix J: AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Foods and Environmental Surfaces.

For each PCR assay and matrix combination, 20 low inoculated (0.2-2 CFU/test portion), five high inoculated (>2 cfu/test portion), and five blank test portions were tested using the PCR assay workflows. The matrix studies followed an unpaired study design where the PCR assay workflows were compared to the applicable reference method. For the SureTect Cronobacter PCR Assay workflow, the reference method used was EN ISO 22964:2017, while for the SureTect Salmonella PCR Assay, the reference method used was EN ISO 6579-1:2017.

The Probability of Detection (POD) was calculated for each matrix, from which the difference in POD (dPOD) between the two workflows was calculated. If the confidence interval of the dPOD did not contain zero, then the difference was statistically significant at the 5% confidence level.

## Matrix study results

Table 2 shows the AOAC POD matrix study results for the PCR assay workflows when comparing to the reference method. The PCR assay workflows met AOAC performance requirements and there were no statistically significant differences observed when comparing to the gold standard reference methods.

**Table 2. POD Results at fractional level for PIF and environmental with *Salmonella* and *Cronobacter* PCR assays.**

| Matrices | Enrichment | SureTect Salmonella PCR Assay | | SureTect Cronobacter PCR Assay | | Result |
|---|---|---|---|---|---|---|
| | | $dPOD_C$[a] | CI[b] | $dPOD_C$[c] | CI | |
| PIF with probiotics 375 g | 1,875 mL pw[d]-BPW with 6 mg/L novobiocin | 0.05 | -0.23, 0.32 | 0.35 | 0.07, 0.57[e] | Pass |
| PIF without probiotics 375 g | 1,875 mL pw-BPW | 0 | -0.28, 0.28 | 0 | -0.28, 0.28 | Pass |
| Stainless steel (1" x 1") | 10 mL BPW | 0 | -0.14, 0.14 | 0.05 | -0.24, 0.33 | Pass |

a. $dPOD_C$: Difference between the confirmed candidate method result and reference method confirmed result POD values. Reference method used was ISO 6579-1:2017.
b. CI (95%): If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
c. $dPOD_C$: Difference between the confirmed candidate method result and reference method confirmed result POD values. Reference method used was ISO 22964:2017.
d. Pre-warmed.
e. Confidence interval did not contain zero due to greater sensitivity of the PCR assay workflow.

## Conclusion and significance

In conclusion, the end-to-end workflows offer a comprehensive and reliable solution for the detection of *Salmonella* and *Cronobacter* species in PIF and related matrices. The PCR assay workflows offer a harmonized enrichment where a single test portion can be tested by both PCR assays. Additionally, the workflows can be run using manual or automated lysis and PCR setup procedures with the CyBio™ FeliX Instrument.[4] This approach provides end users with a cost-effective and efficient testing solution. By incorporating highly reliable PCR assays and user-friendly protocols, these workflows enable laboratories to ensure the safety and quality of PIF products, contributing to the well-being of infants and providing peace of mind to parents and caregivers.

### References


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3. Yang MT, Chi CS. Salmonella infections in infants and children. Zhonghua Yi, Xue Za Zhi (Taipei). 1994 Jul;54(1):38-43. PMID: 8087721. Salmonella infections in infants and children - PubMed (nih.gov) (accessed July 2024)
4. CyBio™ FeliX Instrument for SureTect™ PCR Automation Workflow, https://www.thermofisher.com/order/catalog/product/A66295?SID=srch-srp-A66295 (accessed August 2024)


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