IAFP Latino 2024
Simpósio Latinoamericano em Segurança dos Alimentos
Santos - SP - Brasil
11 a 14 Nov, 2024

Comparison of Hygiena\'s foodproof® Salmonella plus Cronobacter LyoKit and ISO Reference Methods for Validation of Pathogen Detection in Infant Food and Production Environments

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Infections of infants with *Cronobacter* and *Salmonella* species are linked to the consumption of contaminated powdered infant formula (PIF). Therefore, regulatory agencies require screening for these two pathogens throughout the entire production process of PIF. This study evaluated the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit combined with different DNA extraction methods compared to ISO 6579-1 and ISO 22964 standards according to the requirements of DIN EN ISO 16140-2:2016 and the AFNOR Certification technical rules. For the method comparison part of this AFNOR Certification validation study, sensitivity, relative level of detection (RLOD) and specificity studies were conducted by ADRIA Développement. For the sensitivity study, 141 (*Salmonella* target) and 137 (*Cronobacter* target) uncontaminated and artificially contaminated samples of probiotic and non-probiotic infant formula, ingredients (375 g) and production environmental samples (200 g and surfaces) were enriched in buffered peptone water (1:10 dilution; with 10 mg/L vancomycin for probiotic-containing samples) and incubated for 16-20 hours at 37 °C. Following incubation, DNA extraction was performed using the foodproof® StarPrep Three Kit and the BAX® Prep Gram-Negative Lysis Kit, followed by real-time PCR analysis. In addition, 60 samples per target were analyzed by the alternative and the reference methods to determine LOD_{50} and RLOD values. Specificity panels including 100 *Salmonella* and 50 *Cronobacter* strains and 30 non-target strains were evaluated to ensure inclusivity and exclusivity of PCR targets. The method described successfully yielded results comparable to the reference methods. The sensitivity and RLOD results met the acceptability limits for both targets. Specificity studies produce 100% inclusivity for 150 target strains with 100% exclusivity of 30 non-target strains for each target. This validation provides the infant formula industry with a real-time PCR method that simultaneously detects *Salmonella* and *Cronobacter* species from one enrichment culture and in only one PCR reaction.

Agradecimentos: Special thanks to the entire Hygiena R&D and Application team for their contribution