Validation of the Hygiena™ foodproof® *Enterobacteriaceae* plus *Salmonella* Detection PCR LyoKit for Infant Cereals, Infant Formula with or without Supplements, and Production Environmental Samples

Throughout the production of infant formula and cereals, regulatory agencies not only require pathogenic screening for *Salmonella* and *Cronobacter* spp. but also require continued monitoring of the environment for hygienic indicators such as *Enterobacteriaceae*. PCR technologies have improved for pathogens; however, indicator testing lacks advancements in sensitivity, specificity, and time-to-results.

Validation Methods

- The study comprises inclusivity of 53 target strains, and exclusivity of 30 non-target strains, sensitivity, relative level of detection (RLOD) and interlaboratory studies.
- Validation of the alternative method was performed and compared to the cultural reference method for:
  - Infant cereals and infant formula with and without probiotics and other additives (375 g)
  - Production environmental samples (surface swabs, dust samples (200 g))
- Samples were enriched in:
  - Pre-warmed Buffered Peptone Water (1:10)
  - Vancomycin (10 mg/L) addition for products containing B*ifidobacterium lactis, Bifidobacterium longum, Lactobacillus johnsonii* and/or *Streptococcus thermophilus*
  - α-amylase (0.1 g/L) addition for cereal-containing products
  - Incubation for 16 h - 20 h at 37 °C ± 1 °C
- After incubation, DNA extraction was performed with the foodproof StarPrep One Kit or foodproof StarPrep Three Kit (bulk or 8-strip), including a step for live and dead cell differentiation with Reagent D, then lysates were analyzed by Real-Time PCR.

Validation Results

- The MicroVal validation study (certificate No. 2020LR90) indicated that the alternative method performs equally compared to reference methods EN ISO 21528-1:2017 and EN ISO 6579-1:2017/A1:2020 and fulfills the validation criteria according to EN ISO 16140-2:2016.
- The specificity studies yielded:
  - 100% inclusivity of all target strains
  - 100% exclusivity of all non-target strains

Additionally, the acceptability limits for the sensitivity and the relative LOD studies for all categories and enrichment protocols were met.

Industry Significance

- This validation provides a multiplex Real-Time PCR assay that is a rapid and reliable alternative method for the detection of *Enterobacteriaceae* and *Salmonella* in infant cereals, infant formula with or without probiotics and ingredients, and production environmental samples.
- The foodproof assays have a wide range of extraction options and compatible instruments.
- This allows facilities the ability to utilize a single assay to screen for *Salmonella* and *Enterobacteriaceae*, using the same enrichment, lysate, and assay, which improves operational efficiencies throughout food production and environmental monitoring. This also holds true for high throughput testing laboratories.