Development and Internal Validation of Hygiena's foodproof® *Salmonella* plus *Cronobacter* Detection LyoKit

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BAX® System X5 | BAX® System Q7 | foodproof® | microproof®

Introduction

Almost 20 years ago, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) declared that *Salmonella* and *Cronobacter sakazakii* are the microorganisms of greatest concern in powdered infant formula (PIF). Intrinsic contamination of PIF and infant cereals can occur during the manufacturing process, for example, from raw ingredients or from the production environment. Therefore, regulatory agencies require pathogenic screening for *Salmonella* and *Cronobacter* spp. in the final product and at all stages of manufacturing. The recall of PIF in 2022 due to *Cronobacter* and *Salmonella* infections in newborns once again demonstrates the importance of testing infant formula and infant cereals, food products in general, and environmental samples for these two pathogens.

The method is currently being validated according to ISO 16140-2.

Purpose

Development of a rapid real-time PCR assay for the simultaneous detection of *Salmonella* and *Cronobacter* in combination with **food**proof StarPrep® One and Three, **food**proof® Magnetic Preparation Kit I and BAX® System lysis reagents as different options for DNA extraction.

Significance

With the **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit, Hygiena® provides infant formula industries with a rapid, reliable and easy-to-use real-time PCR assay. By combining the detection of *Salmonella* and *Cronobacter* in a single real-time PCR reaction, testing time and costs are significantly reduced. Offering different options for DNA extraction gives the end user a high level of flexibility in choosing a DNA extraction method that meets their individual requirements.

Manufacturer

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foodproof® *Salmonella* plus *Cronobacter* Detection LyoKit

The **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit provides all necessary reagents and a control template for reliable interpretation of results. To improve user experience and kit stability, reagents for the PCR mix are provided lyophilized.

The kit enables the detection of *Salmonella* and *Cronobacter* in a single PCR reaction, with identification of *Salmonella* DNA in the FAM channel and detection of *Cronobacter* DNA in the HEX channel. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to PCR inhibition, this multiplex assay includes an Internal Control (IC) that is simultaneously amplified in the same PCR reaction with the pathogen targets and is detected in the ROX channel. The IC and the control template prove the functionality of the reaction mix for correct amplification of the pathogen targets.

For prevention of carry-over contamination, Uracil-N-Glycosylase is also included in the master mix.

Compatibility with the BAX System

Sixteen samples of three different PIF products with probiotics were examined to evaluate the compatibility of the **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit with the BAX System lysis procedure and the BAX System Q7 instrument. 375 g samples in BPW (1:10 dilution) with vancomycin (10 mg/L) were inoculated with 0.5 and 7.5 CFU of heat-stressed *Salmonella* and *Cronobacter* strains, respectively, and incubated at 37 °C for 16 h, 18 h and 24 h. One sample per PIF product was tested uninoculated. DNA extraction was performed with BAX System lysis reagents using 20 µL enrichment culture. PCR analysis was conducted with 30 µL of DNA extract on the BAX System Q7 instrument.

All inoculated samples tested positive in the respective detection channel for each time point of enrichment. Uninoculated samples tested negative for *Salmonella* and *Cronobacter*. No PCR inhibition due to sample matrix was observed.

These data show that the **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit can be used in combination with the BAX System lysis procedure and the BAX System Q7 instrument.

Sensitivity of the PCR Kit

Sensitivity tests were carried out with purified and quantified DNA of four strains of *Salmonella* and four strains of *Cronobacter* (Table 1). The selected strains cover a broad range of sequence heterogeneity. Dilution series were generated with four concentrations and were analyzed in eight replicates per target on a LightCycler® 480 II instrument. PCR reagents from two different lots were used.

Both target organisms could be detected at a minimal concentration of 5 genome equivalents (GE) of purified DNA.

Sensitivity Including DNA Extraction

Buffered Peptone Water (BPW) with *Escherichia coli* (10^8 CFU/mL) to mimic background flora and enrichment cultures of PIF in BPW (1:10 dilution) were spiked with different concentrations (~10^1 and 10^4 CFU/mL) of *Salmonella* Enteritidis and *Cronobacter sakazakii,* respectively, immediately before DNA extraction (Tables 2a and 2b). DNA isolation was performed manually with the StarPrep One Kit using single-tube and 8-strip formats as well as automatically using magnetic-bead technology, followed by real-time PCR analysis on a LightCycler 480 II instrument.

The limit of detection of the **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit in combination with different DNA extraction methods is 10^2 – 10^3 CFU/mL.

Inclusivity & Exclusivity Studies

Specificity studies were performed by analyzing 141 isolates, including 48 *Salmonella*, 46 *Cronobacter* and 47 non-target strains.

Inclusivity and exclusivity studies demonstrated the specificity of the **food**proof *Salmonella* plus *Cronobacter* Detection LyoKit. All inclusivity organisms were correctly identified in the respective detection channels, while all exclusivity organisms were correctly excluded.

Table 1. Sensitivity of the PCR Kit

GE/Reaction	*Salmonella* Strain	Cp Mean *Salmonella* (FAM)	*Cronobacter* Strain	Cp Mean *Cronobacter* (HEX)
50	*Salmonella* Typhimurium BCD 2210	34.3	*Cronobacter sakazakii* DSM 4485	30.7
20		35.1		31.4
5		37.1		32.5
1		-		33.1
50	*Salmonella* Typhi BCD 7754	33.3	*Cronobacter sakazakii (turicensis)* LMG 2790	30.5
20		34.4		31.4
5		36.0		32.4
1		36.5		33.3
50	*Salmonella enterica* ssp. *arizonae* BCD 14399	33.6	*Cronobacter dublinensis* subsp. *dublinensis* DSM 18705	30.7
20		34.8		31.6
5		35.3		32.5
1		-		33.2
50	*Salmonella bongori* BCD 5242	33.1	*Cronobacter muytjensii* ATCC 51329	30.5
20		34.0		31.4
5		36.7		32.4
1		37.5		33.1

Table 2a. Sensitivity Including DNA Extraction - PIF samples

Spiking Level (CFU/mL)	Cp Mean *Salmonella* (FAM)			Cp Mean *Cronobacter* (HEX)		
	StarPrep One Kit		Magnetic Preparation Kit I	StarPrep One Kit		Magnetic Preparation Kit I
	Single Tube	8-strip Tube		Single Tube	8-strip Tube	
10^4	28.4	29.4	32.8	27.2	28.5	30.8
10^3	31.6	32.6	35.6	30.4	31.7	33.2
10^2	34.4	35.6	38.1 / -	32.9	33.9	34.5
10^1	37.4	- / 36.5	- / 38.2	34.5	34.4 / -	-
unspiked	-	-	-	-	-	-

Table 2b. Sensitivity Including DNA Extraction - BPW + *E. coli* (10^8 CFU/mL) samples

Spiking Level (CFU/mL)	Cp Mean *Salmonella* (FAM)			Cp Mean *Cronobacter* (HEX)		
	StarPrep One Kit		Magnetic Preparation Kit I	StarPrep One Kit		Magnetic Preparation Kit I
	Single Tube	8-strip Tube		Single Tube	8-strip Tube	
10^4	27.2	29.3	31.8	25.5	27.7	30.3
10^3	29.6	32.2	33.4	28.8	30.3	32.6
10^2	31.6	35.3	35.5	31.4	33.5	33.7
10^1	34.4	-	35.7 / -	32.3 / -	33.8 / -	34.1 / -
unspiked	-	-	-	-	-	-