Detection of *Salmonella* from 375 g Powdered Infant Formula Using the Hygiena® BAX® System Real-Time PCR Assay

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INTRODUCTION:

Powdered infant formula (PIF) is not a sterile product, and despite stringent manufacturing controls, it can become contaminated with foodborne pathogens like *Salmonella* or *Cronobacter sakazakii*. These organisms have been implicated in both outbreaks and sporadic cases of illness in infants (1, 2).

Given the vulnerability of infants and the widespread use of PIF as a primary or supplemental nutritional source, ensuring its microbiological safety is critical.

PURPOSE:

This study was designed to evaluate the performance of a commercial real-time PCR assay compared with the US FDA BAM reference method for the detection of *Salmonella* in 375 g test portions of powdered infant formula, with an optional secondary 3-hour regrowth.

REGISTERED TRADEMARKS:

BAX® is a registered trademark of Hygiena® which offers equipment, reagents and software for analyzing samples for microbial contamination.
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METHODS:

Paired test portions (375 g) of a milk-based powdered infant formula were inoculated with a heat-stressed *Salmonella* Agona culture to create two levels of contamination: a low level expected to yield fractional positive results (n=20) and a high level expected to yield all positive (n=5). Five additional samples were left uninoculated as controls. Following inoculation, samples were stabilized at room temperature for 2 weeks prior to the start of enrichment.

Samples were homogenized with 3,375 mL of Lactose Broth (LB) and held at room temperature for 55-65 minutes. The pH was measured to ensure the enrichment was 6.8 ± 0.2. Samples were then incubated at 35 °C for 22-26 hours. Aliquots were removed and tested by real-time PCR directly from the primary enrichment and with a secondary 3-hour BHI regrowth.

All samples were confirmed by culture, according to the FDA BAM Chapter 5: *Salmonella* (Updated May 2024).

RESULTS:

Test method
Real-Time PCR presumptive positive results for:
- 14/20 low inoculated samples
- 5/5 high inoculated samples
- 5 uninoculated controls were negative
- Identical results obtained directly from LB enrichment and with a 3-hour BHI regrowth.

Reference method
Confirmed positive results for:
- 14/20 low inoculated samples
- 5/5 high inoculated samples
- 5 uninoculated controls were negative

Statistical significance (Table 1)
- No significant differences were observed when comparing the test and reference method results using the probability of detection (POD).

SIGNIFICANCE:

The study results demonstrate that the BAX® System Real-Time PCR assay is sensitive, specific, and accurate for the detection of *Salmonella* in 375 g samples of powdered infant formula in 22-26 hours.



TABLE 1: Test Method Results vs. Reference Method Results

Target Strain	MPN/Test Portion	N	BAX System Method			Reference Method			$dPOD_C$	95% CI
			X	POD_C	95% CI	X	POD_R	95% CI		
Salmonella **Agona HCC 13743**	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
	1.2	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0.00	-0.27, 0.27
	10.1	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.43, 0.43

MPN/Test Portion = Most Probable Number is based on the POD of reference method test portions, **N** = Number of test portions, **X** = Number of positive test portions, **POD_C** = Confirmed BAX System method positive results divided by the total number of test portions, **POD_R** = Confirmed reference method positive results divided by the total number of test portions, **$dPOD_C$** = Difference between the BAX System method and reference method POD values, **95% CI** = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

REFERENCES:

1. Cahill, S. M., K. Wachsmuth, M. de L. Costarrica, P. Karim, B. Embarek. 2008. Powdered infant formula as a source of *Salmonella* infection in infants. Clin Infect Dis. 46(2):268-273.
2. Kent, R. M., G. F. Fitzgerald, C. Hill, C. Stanton, R. P. Ross. 2015. Novel Approaches to Improve the Intrinsic Microbiological Safety of Powdered Infant Milk Formula. Nutrients. 7(2):1217-1244.

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