



One Health Diagnostics™

Detection of *Salmonella* from 375 g Dry Kibble Pet Food in 16 Hours Using Hygiena's BAX® System Real-Time PCR Assay

Micah Greenzweig, Julie Weller, Ilir Mandija
Hygiena®, 2 Boulden Circle, New Castle, DE 19720

BAX® System Q7

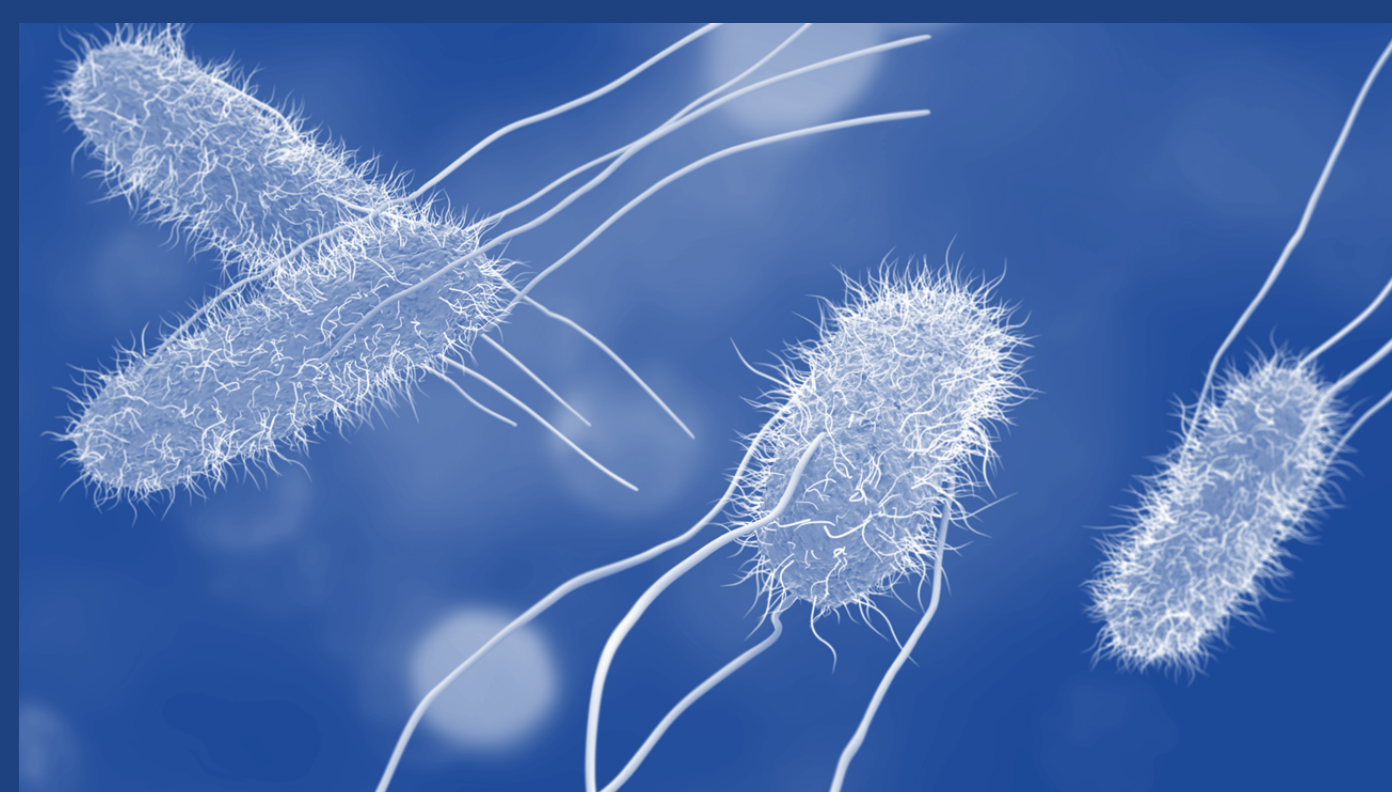
BAX® System X5

foodproof®

microproof®

INTRODUCTION:

Pet food and pet treats are well-established vehicles for *Salmonella* contamination. These products can transmit the pathogenic bacteria to both companion animals and humans causing salmonellosis. In 2021, the Food and Drug Administration (FDA) reported at least 10 recalls of pet food or pet treats due to the potential presence of *Salmonella* (1).



PURPOSE:

This study was designed to evaluate the performance of a commercial real-time PCR assay compared to the ISO reference standard for the detection of *Salmonella* from paired 375 g test portions of dry kibble dog food.

REGISTERED TRADEMARKS

BAX® is a registered trademark of Hygiena® for its line of equipment, reagents and software used to analyze samples for microbial contamination. Hygiena® is a registered trademark of Hygiena.

METHODS:

Test portions were inoculated with *Salmonella houtenae* at a low level to create 20 samples expected to yield fractional positive results and at a high level to create 5 samples expected to be all positive. An additional 5 samples were left uninoculated as controls. Following inoculation, samples were equilibrated at ambient laboratory conditions for 2 weeks.

Samples were enriched following ISO 6579-1:2017 *Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of Salmonella*. Paired samples were tested at 16 hours by real-time PCR and confirmed by culture.

RESULTS:

Real-time PCR (16 h)

- Fractional recovery achieved in the low inoculation level (8/20 positives or 40%)
- All 5/5 high-inoculated samples were positive (100%)
- All uninoculated controls were negative
- No difference in results when processed with or without a 3-hour BHI regrowth

Culture (24 h)

- All real-time PCR results were identical to culture with 100% agreement.
- No false positives or false negatives

No statistical difference between real-time PCR and the reference method (Table 1).

SIGNIFICANCE:

The study results demonstrate that the BAX® System Real-Time PCR assay is sensitive, specific and accurate for the detection of *Salmonella* species in 375 g samples of dry kibble dog food in 16-24 hours.



Table 1. BAX System Method vs. Reference Method Results

Sample Type	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		
Dry Dog Food (375 g)	<i>Salmonella houtenae</i> DD13282	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	0.00, 0.00
		0.52	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
		7.05	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. US Food & Drug Administration (US-FDA). Recalls & Withdrawals. <https://www.fda.gov/animal-veterinary/safety-health/recalls-withdrawals>.
2. US Food & Drug Administration (US-FDA). October 24, 2011. FY 2012 National Assignment to Collect and Analyze Samples of Pet Foods, Pet Treats, and Supplements for Pets from Interstate Commerce in the United States for *Salmonella*. <https://www.fda.gov/animal-veterinary/biological-chemical-and-physical-contaminants-animal-food/fy-2012-nationwide-assignment-collect-and-analyze-samples-pet-foods-pet-treats-and-supplements-pets>.