Evaluation of the EnviroLogix™ DNAble® *Salmonella* DNA Detection Kit using Dry Dog Food

DATA SUMMARY

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INTRODUCTION

OBJECTIVES:

To assess 1) the feasibility of 1:4 and 1:10 mBPW volumes using 375g samples of dry dog food, 2) the compatibility of the DNAble assay in 10 types of dry dog food and 3) to demonstrate the compatibility of the DNAble assay to the BAM using a stressed *Salmonella* cell spike.

METHODS:

Objective1: Fourteen 375g portions of a single brand of dry dog food were weighed out. 1.5 L of mBPW was added to each of 7 samples (1:4), 3.75 L was added to each of 7 samples (1:10). 3 of the 1:4 and 1:10 samples were inoculated with approximately 2 cells (low spike)of *Salmonella enterica enteritidis* (SEE), similarly 3 of each of the 1:4 and 1:10 samples were inoculated with approximately 5 cells (high spike) of SEE and one of each sample were not inoculated. All samples were incubated overnight at 37°C, prepared and tested via DNAble. Culture confirmation as per FDA-BAM was also performed.

Objective2: 75 mL of mBPW was added to 25 g of 10 different dry dog food types, representing diverse quality and the quantity of ingredients, which were then inoculated with an estimated 10 cells of SEE and incubated overnight at 37°C. Following incubation, these samples where then prepared and run through the DNAble assay.

Objective 3: A low and high cell count of heat-stressed SEE was spiked onto 25g samples of dry dog food. The samples were allowed to sit for 5 days and were then suspended with 225 mL of mBPW, incubated at 37°C overnight, processed and evaluated for the presence of *Salmonella* using DNAble and culture.

RESULTS:

Objective 1: All DNAble and culture results were as expected with low and high SEE cell spike giving positive results and the non-inoculated sample giving negative results.

Objective 2: All results were as expected with all 10 types of dog foods producing positive DNAble results.

Objective 3: All results were as expected with the low cell count (near LOD) inoculated samples yielding partial positive results and the high cell count inoculated samples yielding all positive results with DNAble and culture.

METHODS

Objective 1: 375g portions of unground dog food (whole kibbles) were weighed and placed in stomacher bags for a 1:4 dilution (1.5 L mBPW added) and into 4L bottles for the 1:10 dilution (3.75 L mBPW added). 3 samples from each dilution level were inoculated with approximately 2 cells (low spike) of *Salmonella enterica enteritidis* (SEE) and 3 samples from each dilution were inoculated with approximately 5 cells (high spike) of SEE. One sample from each dilution was left un-inoculated as a negative control. All samples were then incubated overnight at 37°C. The following day, all samples were thoroughly hand massaged and prepared using the following protocol:

1. Add 1 mL of enriched sample to a microcentrifuge tube and centrifuge at 10,000g for 5 minutes
2. Remove supernatant and resuspend pellet in 100µL of MB3 buffer
3. Place in 95°C heat block for 10 minutes
4. Centrifuge heated sample at 10,000g for 5 minutes
5. Use 5 µL of heated sample for DNAble

Culture confirmation was performed using FDA-BAM Chapter 5 as a reference.

Objective 2: 25 g of 10 different dry dog foods was measured into filtered stomacher bags. 75 mL of mBPW was then added to each bag. Each bag was inoculated with an estimated 10 cells of SEE followed by thorough hand mixing. The samples were incubated overnight at 37°C. The following day the samples were hand mixed for uniform pasty consistency and liquid was collected on the filtered side of the bags and was processed for DNAble using the protocol the following protocol:

1. Add 1 mL of enriched sample to a microcentrifuge tube and centrifuge at 10,000g for 5 minutes
2. Remove supernatant and resuspend pellet in 100µL of MB3 buffer
3. Place in 95°C heat block for 10 minutes
4. Centrifuge heated sample at 10,000g for 5 minutes
5. Dilute supernatant 1:5 in MB3 buffer
6. Use 5 µL of heated sample for DNAble

Objective 3: 100 cells (low spike) and 10^6 (high spike) of heat-stressed SEE were inoculated onto 25g dry dog food samples in Whirlpak® filter bags. For heat stress the SEE cells were heated at 50˚C for 10min followed by the plate count determination. The samples were allowed to sit at room temperature for 5 days after which 225 mL of mBPW were added. Following an overnight at 37°C, the samples were prepared using the protocol detailed above in Objective 2. *Salmonella* detection was performed using DNAble. Overnight enrichments were also inoculated to XLT4 media for confirmation of *Salmonella* (black colonies). Discordant analysis (DNAble +/XLT4 -) was conducted by re-enriching the overnight enriched samples in TT broth and with subsequent plating to XLT4 as well as repeat DNAble testing.

RESULTS

Objective 1: Both culture volumes appear to be compatible with DNAble. Regardless of culture volumes used, the DNAble was able to detect *Salmonella* in the dog food used in this study. Furthermore, spike-in levels did not affect the outcome (see **Table 1** below).

Matrix Amt	Sample: Media	Spike levels (CFU/ portion)	DNAble Result	BAM Culture Result
375g	1:4	No spike in	Neg	Neg
		0.2 -2	Pos	Pos
			Pos	Pos
			Pos	Pos
		2-5	Pos	Pos
			Pos	Pos
			Pos	Pos
375g	1:10	No spike in	Neg	Neg
		0.2 - 2	Pos	Pos
			Pos	Pos
			Pos	Pos
		2-5	Pos	Pos
			Pos	Pos
			Pos	Pos

Objective 2: Although there was variability in the amplification curves of *Salmonella* in the 10 different dry dog foods, all were considered positive by the DNAble algorithm. (See **Figures 1 and 2**)

Figure 1
Samples 1-8 and 11-16 represent 7 types of dog food while samples 9-10 are negative controls.

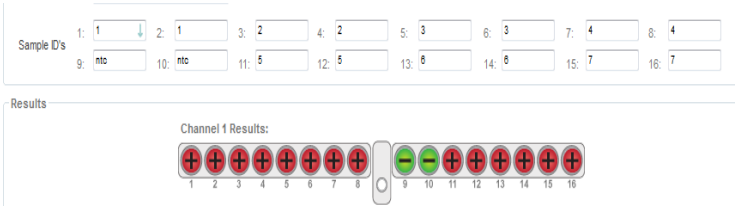
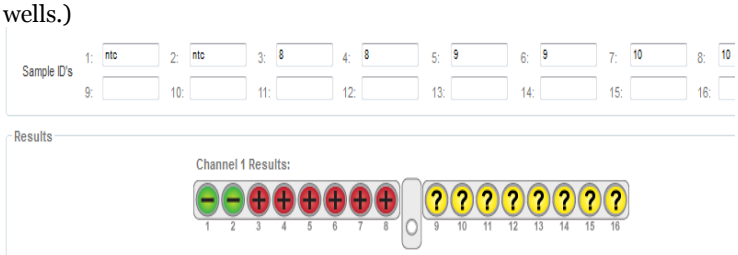


Figure 2
Samples 3-8 represent 3 additional types of dog food while samples 1-2 are negative controls. (Samples 9-16 are empty wells.)



Objective 3: All results were as expected with the high cell count spiked samples yielding positive DNAble as well as positive culture results. The low cell spiked samples performed as expected giving partial positive results which is typical for testing close to the Limit of Detection of the assay (See **Table 2**).

Table 2

	Low Spike			High Spike		
DNAble*	-/+	+/+	+/+	+/+	+/+	+/+
Culture	neg	pos	neg	pos	pos	pos
TT re-enrichment**	neg	ND	neg	ND	ND	ND
DNAble re-re-run	neg	ND	neg	ND	ND	ND

***Represents results from two replicates per treatment**

****ND: Not done**

CONCLUSION:

The DNAble kit described in this study provides accurate and rapid detection of *Salmonella* in dry dog food as compared to culture. The reader is simple to use and requires a minimal footprint. Sample preparation is simple and results are available approximately 40 minutes after an overnight enrichment.