Evaluation of the EnviroLogix® DNAble® *Salmonella* DNA Detection Kit for Poultry Pre-harvest Samples

DATA SUMMARY

A. DAVIS AND V. DUTTA

ABSTRACT

OBJECTIVE: To compare the performance of the DNAble® *Salmonella* DNA Detection Kit to culture for the detection of *Salmonella* in poultry environmental samples (boot swabs, drag swabs, chick papers) collected from poultry production sites.

METHODS: More than 300 environmental samples were evaluated for the presence of *Salmonella* DNA using the DNAble® methodology and culture. The DNAble *Salmonella* DNA Detection Kit utilizes an isothermal nucleic amplification technology enabling rapid amplification of a specific DNA target. After collection and processing, the samples are added to reaction buffer. The reaction buffer containing the sample is then transferred to the lyophilized master mix. Results are obtained in 15 minutes using the DNAble Reader.

Testing was performed at 3 different laboratories in the United States. Samples were initially placed in BPW for 15 minutes, stomached, and were then evenly divided for comparison of DNAble to culture.

RESULTS: The DNAble® performance compared favorably with culture showing proposed resolved sensitivity and specificity of 96.1% and 99.1% respectively against the culture pathway.

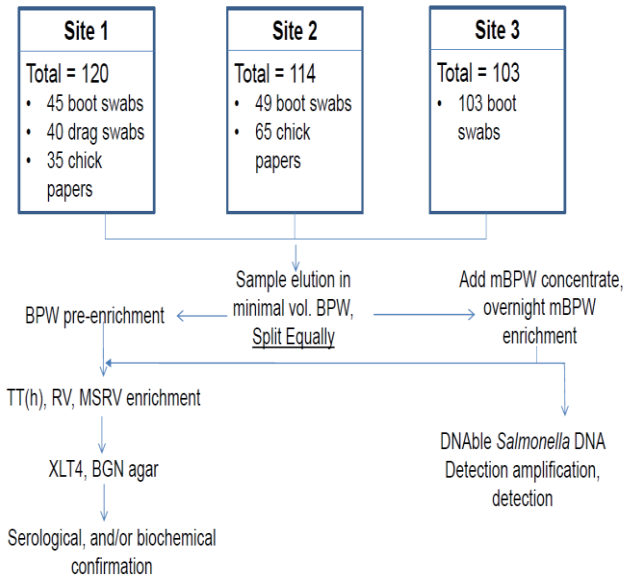
METHODS

Sites analyzed samples submitted to their respective laboratories using culture and DNAble. Samples were environmental samples from poultry producers which included boot swabs, drag swabs and chick papers.

At all sites, the sample volume dedicated to culture was diluted with 9 additional volumes of BPW and incubated at 37°C for 22-24 hours. The resulting culture was used to inoculate tetrathionate broth (TT) using a 10 fold dilution, Rapport-Vassiliadis broth (RV) using a 100 fold dilution and a modified semi-solid RV plate (MSRV) with 0.1 mL. All three subcultures were incubated at 42°C for 22-24 hours. These cultures were then plated to xylose lysine tergitol-4 (XLT4) agar and brilliant green agar with novobiocin (BGN) followed by incubation at 37°C for 24-48 hours. In most cases, colonies suspicious for *Salmonella* were subcultured to non-selective media. *Salmonella* identification was then made using either the Vitek® Microbial Identification System or serological reagents (See **Figure 1**).

DNAble selective *Salmonella* enrichment, sample processing, and amplification/detection were performed per the manufacturer's instructions. The presence of *Salmonella* in DNAble enriched cultures was confirmed following methods described for the comparator *Salmonella* culture pathways.

Figure 1



RESULTS

The performance of the DNAble® *Salmonella* DNA Detection Kit for Poultry Pre-Harvest Samples was compared to culture. Overall assay performance for all three sites after resolution of discrepant results was sensitivity, specificity and overall accuracy of 96.1%, 99.1% and 98.2% respectively (See **Table 1**).

Discordant resolution: At Site 1, 2 out of 44 samples were DNAble® false negative compared to culture and 2 out of 76 were false positive. Discordance was not resolved. At Site 2, 2 out of 28 were false negative by DNAble® and this discordance was unresolved. 3 out of 86 were initially false positive by DNAble® as compared to BPW culture. However, Salmonella was isolated and identified from culture of the mBPW from 2 of these samples. The third false positive was unresolved. At Site 3, 6 out of 28 were initially DNAble® false negative when compared to culture. This was determined to be a technical error by the site and upon retesting retains in-house, all results were positive. No false positive results were reported. (See **Table 2**)

Table1 Combined Data Sites 1-3 (n=337)

	Unresolved	Resolved
%Sensitivity	90.0 (90/100)	96.1 (98/102)
%Specificity	97.9 (232/237)	99.1 (233/235)
%Accuracy	95.5 (322/337)	98.2 (331/337)

Table 2 Sample Breakdown Sites 1-3

Sample Type	Unresolved %Sensitivity	Unresolved %Specificity	Unresolved %Accuracy
Boot Swab	90.1 (73/81)	99.1 (115/116)	95.4 (188/197)
Drag Swab	89.5 (17/19)	100.0 (21/21)	95.0 (38/40)
Chick papers		98.0 (98/100)	98.0 (98/100)
	Resolved %Sensitivity	**Resolved %Specificity**	**Resolved %Accuracy**
Boot Swab	97.6 (80/82)	99.1 (114/115)	98.5 (194/197)
Drag Swab	89.5 (17/19)	100.0 (21/21)	95.0 (38/40)
Chick papers	100 (1/1)	98.0 (97/99)	98.0 (98/100)

CONCLUSION

The DNAble® kit described in this study provides rapid, sensitive, specific and accurate detection of *Salmonella* comparable to existing methods. The reader is simple to use, is portable and requires a minimal footprint. Sample preparation time is minimal. Results are available 40 minutes after an overnight enrichment making turnaround time considerably more rapid than traditional culture.

SUPPLEMENTAL PERFORMANCE INFORMATION

Analytical Reactivity:

A broad recognition of a variety of *Salmonella enterica* serotypes was demonstrated by detection after mBPW culture and DNAble detection or amplification/detection of DNA purified from isolates. See **Table 3** below.

Table 3

mBPW & Amplification:	Amplification using purified DNA:
Salmonella Typhimurium	*Salmonella* Typhimurium
Salmonella Gallinarum	*Salmonella* Typhi
Salmonella Paratyphi	*Salmonella* Kentucy
Salmonella Newport	*Salmonella* Saintpaul
Salmonella Mbandaka	*Salmonella* Arizonae
Salmonella Senftenberg	*Salmonella* Dublin
Salmonella Enteritidis	*Salmonella* Gallinarum
Salmonella Heidelberg	*Salmonella* Choleraesuis
Salmonella Montevideo	*Salmonella* Pullorum
Salmonella Pullorum	*Salmonella* Paratyphi B
Salmonella Anatum	*Salmonella* Schwarzengrund
Salmonella Muenchen	*Salmonella* Paratyphi A
Salmonella Schwarzengrund	*Salmonella* Enteritidis
Salmonella Lixington	*Salmonella* Newport
Salmonella Adelaide	*Salmonella* Heidelberg
Salmonella Tennessee	*Salmonella* Infantis
Salmonella Ealing	*Salmonella* Dublin
Salmonella Worthington	*Salmonella* 1,4, (5), 12:i:-
Salmonella Newport	*Salmonella* Paratyphi C
Salmonella Idikan	*Salmonella* Hadar
Salmonella Rissen	*Salmonella* Javiana
Salmonella Isangi	*Salmonella* Arizonae
Salmonella Panama	*Salmonella* Diarizonae
Salmonella Give	
Salmonella Saintpaul	
Salmonella Agona	
Salmonella Livingstone	
Salmonella Yoruba	
Salmonella Cerro	
Salmonella Infantis	
Salmonella Bredeney	
Salmonella Ohio	
Salmonella Norwich	
Salmonella Johannesburg	
Salmonella Branderup	
Salmonella Muenchen	
Salmonella Abaetetuba	

Cross reactivity Studies:

A number of non-*Salmonellae* bacteria were used to challenge the assay using culture and DNAble or by amplification and detection of pure DNA. None were detected. **See Table 4** below.

Table 4

Culture & Amplification:	Purified DNA:
Citrobacter sp.	*Listeria monocytogenes*
Pseudomonas sp.	*Shigella boydii*
Proteus sp.	*Enterobacter aerogenes*
Enterobacter sp.	*Yersinia enterocolitica*
	Citrobacter freundii
	Escherichia coli
	Shigella dysenteriae
	Campylobacter jejuni
	Proteus vulgaris
	Klebsiella pneumonia
	Vibrio sp.
	Clostridium sp.
	Bacillus sp.
	Staphylococcus aureus