Technical Bulletin: Evaluation of the BAX® System Real-Time and Standard PCR Assays for the Detection of *Salmonella* spp. in Boot Swabs

A paired validation was conducted to evaluate the performance of the BAX® System against culture for the detection of *Salmonella* in boot swabs collected from poultry production sites. Samples in this study were assessed for natural contamination using the National Poultry Improvement Plan (NPIP) approved pre-enrichment procedure, followed by rapid screening with the BAX® System or selective enrichment and plating. The BAX® System results were compared to culture demonstrating sensitivity, specificity and overall accuracy of 100%. These results provide the poultry industry with a rapid, highly sensitive PCR-based method equivalent to culture for on the farm detection of *Salmonella.*

Introduction

Salmonella commonly infects poultry and is extensively found in primary production facilities. Since the organism is shed in feces, boot swab sampling provides an easy collection system to assess the prevalence of *Salmonella* in flocks (1, 2). If found positive, the appropriate control measures and sanitation procedures can be applied to decrease and prevent contamination during processing. PCR screening methods are a fast and effective option for detecting *Salmonella* in such sample types.

Sample Preparation and Enrichment

Boot swabs (n=24) acquired from an industry partner were screened for the presence of naturally occurring *Salmonella*. Each boot swab was enriched with 100 mL of pre-warmed (37°C) Buffered Peptone Water (BPW) and incubated at 37°C for 22-24 hours. Sample aliquots were removed at 22 hours and tested by the BAX® System with and without a secondary 3-hour BHI regrowth.

In parallel, 1 mL and 0.1 mL aliquots of the pre-enriched BPW samples were transferred into 10 mL of Tetrathionate (TT) broth and 10 mL of Rappaport-Vassiliadis (RV) broth, respectively. Selective enrichments were incubated at 42°C for 22-24 hours. Sample aliquots from TT were removed at 24 hours and tested by the BAX® System method with a secondary 3-hour BHI regrowth.

Method

BAX® System Method

All samples were processed following the procedures for the BAX® System Real-Time *Salmonella* (KIT2006) and *Salmonella* 2 (KIT2011) PCR assays described in the BAX® System Q7 User Guide.

Reference Method

All samples were culture confirmed regardless of BAX® System results following the procedures in the National Poultry Improvement Plan Program Standards (NPIP, December 2019) using selective and differential plating.

Results

For BPW enrichments, both BAX® System PCR assays (Real-Time *Salmonella* and *Salmonella* 2) returned positive results for 5/24 samples at 22 hours. Results were in complete agreement when tested with and without a 3-hour BHI regrowth.

Similarly, for TT enrichments, both PCR assays returned positive results for 5/24 samples after using a BHI regrowth. All BAX® System results were identical to culture.

To compare the results between the BAX® System and culture, multiple test statistics were calculated including sensitivity, specificity and accuracy (Table 1). All of these measurements were determined to be 100%. In addition, the false negative rate and false positive rate were calculated to be 0%.

Conclusions

Overall, this study provides accurate and reliable results using the BAX® System Real-Time PCR assay for *Salmonella* and the BAX® System PCR assay for *Salmonella* 2 to rapidly screen boot swabs for the presence of *Salmonella* using the following protocol (Figure 1):

- Enrich 1 boot swab with 100 mL of pre-warmed (37°C) BPW and incubate at 37°C for 22-24 hours. Prepare samples for the BAX® System.

- Transfer 1 mL of the pre-enriched BPW sample to 10 mL of TT broth and incubate at 42°C for 22-24 hours. Perform a secondary regrowth by transferring 10 µL of the enriched TT into 500 µL of pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. Prepare samples for the BAX® System.

References

1. Mallinson, E. T., Tate, C. R., and Miller, R. G. (1989). Monitoring Poultry Farms for *Salmonella* by Drag-Swab Sampling and Antigen-Capture Immunoassay. Avian Diseases. 33(4):684-690.
2. St. Amand, J. A., Cassis, R., King, R. K., and Annett Christianson, C. B. (2017). Prevalence of *Salmonella* spp. in environmental samples from table egg barns in Alberta. Avian Pathology. 46(6):594-601.

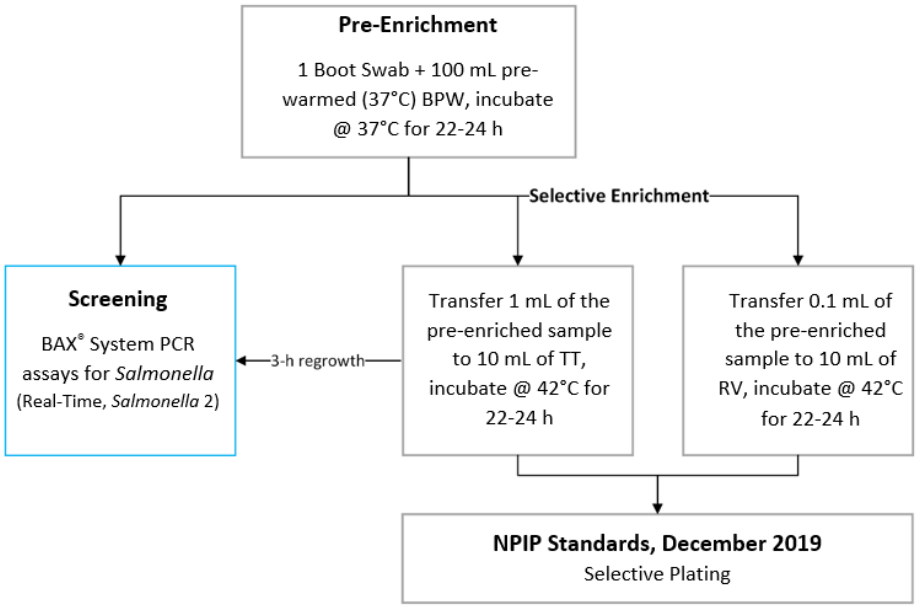

Figure 1: Paired validation study to compare the BAX® System method to culture for boot swabs

Table 1. Paired Study Results for *Salmonella* tested by the BAX® System and Culture

Sample Type	Enrichment	N	BAX® System Positive	Culture Confirmed	Sensitivity	FN Rate	Specificity	FP Rate	Accuracy
Boot Swabs	BPW (22 h)	24	5	5	100%	0%	100%	0%	100%
	BPW (22 h) – TT (24 h) + BHI regrowth	24	5	5	100%	0%	100%	0%	100%

N= Number of test portions.

Sensitivity = 100 times the number of true BAX® System positive results divided by the total true positive results confirmed by culture.

FN Rate = False negative rate is 100 minus the sensitivity rate.

Specificity = 100 times the number of BAX® System negative results divided by the total number of true negative results.

FP Rate = False positive rate is 100 minus the specificity rate.

Accuracy = 100 times the number of BAX® System positives plus the number of BAX® System negatives divided by the total number of samples.