Decreasing the Confirmation Time for *Salmonella* and *Listeria* Using an Alternative Procedure Coupled with Hygiena's BAX® System

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INTRODUCTION:

Culture procedures for confirming a presumptive positive screening result can take several days to complete. After the initial positive result, the target organism is isolated using secondary selective enrichments and/or plating agars to obtain suspect colonies. Then, further biochemical tests and serology are required for identification.

PURPOSE:

The purpose of these studies was to evaluate an alternative confirmation protocol for *Salmonella* and *Listeria* initiated directly from the primary enrichment to confirm presumptive positive results with a quicker turn-around-time.

REGISTERED TRADEMARKS

METHODS:

Enrichments:

Four matrices, including environmental sponges, pasteurized liquid egg whites and whole eggs, and dried whole egg powder, were enriched according to validated protocols for *Salmonella* and *Listeria* (Table 1). After incubation, aliquots were inoculated with various *Salmonella* or *Listeria* cultures at 10^4 CFU/mL, 10^5 CFU/mL and 10^6 CFU/mL, expected to be all positive. Post-inoculated enrichments were screened with BAX® Real-Time PCR and confirmed by culture.

Confirmation:

Samples were confirmed following two procedures.

1. Alternative confirmation procedure with additional colony testing using the BAX System Real-Time PCR assays.
2. Traditional confirmation procedures in the USDA FSIS reference methods.
   - *Salmonella* agars include XLD, DMLIA and Brilliance™ *Salmonella*
   - *Listeria* agars include MOX and PALCAM

RESULTS:

Salmonella:

- Real-time PCR: Consistent positive results for all matrices and sample sizes.
- Alternative confirmation: Primary (BPW) enrichments were directly plated onto all *Salmonella* agars resulting in typical colonies. Colonies were screened with real-time PCR to confirm as positive.
- Traditional confirmation: Secondary enrichments (TT and RV) were plated onto all *Salmonella* agars resulting in typical colonies.

Listeria:

- Real-time PCR: Consistent positive results for all matrices and sample sizes.
- Alternative confirmation: Primary enrichments were directly plated onto all *Listeria* agars resulting in typical colonies. Colonies were screened with real-time PCR to confirm as positive.
- Traditional confirmation: Secondary enrichments (MOPS-BLEB) were plated onto all *Listeria* agars resulting in typical colonies.

SIGNIFICANCE:

Overall, the results demonstrate equivalent performance between the alternative confirmation procedures and the USDA-FSIS reference culture methods to isolate *Salmonella* and *Listeria*. Furthermore, colony testing with the BAX System can shorten the confirmation time.

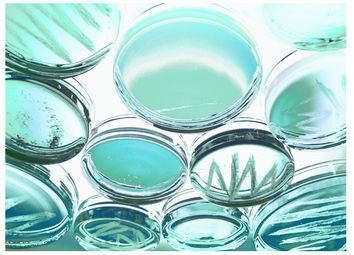

Table 1: Enrichment Protocols

Salmonella

Matrix	Protocol
Environmental Sponge	90 mL pre-warmed BPW, 18-24 h @ 35 °C
Pasteurized Liquid Egg Whites (375 g) Pasteurized Liquid Whole Egg (375 g)	1,500 mL pre-warmed BPW, 20-24 h @ 35 °C
Pasteurized Liquid Egg Whites (100 g) Pasteurized Liquid Whole Egg (100 g) Dried Whole Egg Powder (100 g)	900 mL pre-warmed BPW, 18-24 h @ 35 °C
Dried Whole Egg Powder (375 g)	3,375 mL pre-warmed BPW, 18-24 h @ 35 °C

Listeria

Matrix	Protocol
Environmental Sponge	90 mL pre-warmed 24 LEB Complete, 24-48 h @ 35 °C 90 mL pre-warmed Actero™ Listeria, 24-48 h @ 35 °C
Pasteurized Liquid Egg Whites (125 g) Pasteurized Liquid Whole Egg (125 g)	1,125 mL pre-warmed 24 LEB Complete, 24-48 h @ 35 °C
Pasteurized Liquid Egg Whites (25 g) Pasteurized Liquid Whole Egg (25 g) Dried Whole Egg Powder (25 g)	225 mL UVM, 24 h @ 30 °C. Secondary transfer MOPS-BLEB 18-24 h @ 35 °C
Dried Whole Egg Powder (125 g)	1,125 mL pre-warmed 24 LEB Complete, 48 h @ 35 °C