Detection of Salmonella Enteritidis in Egg and Poultry Samples Using a New Lateral Flow Immunoassay

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MATERIALS AND METHODS

INOCULUM STRAINS

Raw shell eggs, chicken carcass rinses, and poultry feed were inoculated with different strains of SE. Inoculum strains are shown in Table 1. Poultry house environmental samples were tested without inoculation. Details of inoculation and sample preparation for the various matrices follow.

RAW SHELL EGGS

Eggs were surface disinfected with a 3:1 mixture of 70% isopropanol and an indole/phenol solution in accordance with the FDA/BAM reference method. Eggs were cracked and a pool of 1,400 eggs prepared. 10 L was withdrawn to serve as unincriminated control broth (UCC). Another 10 L was inoculated at a level of 1/2,000,000 (0.5% SE) and served as positive control broth (PCB). It was reserved for MPN analysis. The remaining eggs were resuspended for the fractional level inoculation. Eggs were inoculated with a dilution of an overnight culture at the following levels: high – 15 cfu/egg, fractional – 0.5% DNA/egg. Inoculated and control eggs were divided into 20-egg portions (1 L each) and held at 2-4°C for 48-72 hours. Forty test portions were prepared at the fractional level, 10 at the high level, and 10 controls. Half of the test portions were analyzed with the Reveal method and the other half with the appropriate reference method.

For the Reveal method, 400 mL Reveal 2.0 for SE Medium and 2 mL Reveal 2.0 for SE Supplement were added, and the samples incubated at 35°C for 24 hours. Following the primary inoculation, 0.1 mL of the culture was transferred to a tube containing 2.5 mL of MRS, and incubated at 35°C for 48-72 hours.

For the FDA/BAM reference method, test portions held at 2-4°C were incubated at room temperature for 96 hours. Analysis was continued in accordance with the published method.

RESULTS

For the raw shell egg, chicken carcass rinse, and poultry feed study shown in Table 1. There were no significant differences in the number of results by the Reveal and reference methods for any of the commodities tested as determined by chi-square analysis (P > 0.05). Considering all data, there were 53 confirmed positive results by the Reveal method and 50 by the reference culture procedures. There was a single unconfirmed positive result on a fractional-level chicken rinse test portion; otherwise, all positive Reveal results were confirmed by plating from the MRS secondary enrichment.

Results of poultry house environmental sample testing from third-party laboratories are shown in Table 2. There were a total of 636 samples processed in a paired design (common primary enrichment). There were 85 positives by the Reveal method, of which 64 were confirmed independently from the MRS tube, ex. 62 by the MPR reference method. Confirmation of SE was especially difficult in laboratory 3, for both the Reveal and MPR reference methods, as these samples were heavily co-contaminated with group E4 Salmonellae. The use of the SE immunomagnetic beads added the confirmation process, but did not resolve all cases of unconfirmed Reveal results. It is noteworthy that sensitivity of the Reveal method was 100%, there was no Reveal-negative, NPIP reference method-positive results.

DISCUSSION AND CONCLUSIONS

Results presented here show the Reveal 2.0 for SE test can be used as a reliable alternative to reference culture methods for detection of SE in a wide variety of poultry-associated matrices. For the diverse group of sample types, ranging from raw shell eggs to poultry house environmental swabs, the Reveal method was as productive as the respective reference culture method in recovery and detection of SE, while providing results within 48 hours.

REFERENCES


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