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Lansing, Michigan USA

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

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ABSTRACT

A new lateral flow immunoassay, Reveal® 2.0 for *Salmonella* Enteritidis (SE), has been developed and validated for detection of SE in raw shell eggs, chicken carcass rinse, poultry feed, and poultry house environmental samples. The new Reveal 2.0 method features a novel enrichment procedure utilizing primary enrichment followed by secondary enrichment in a tube containing modified semi-solid Rappaport-Vassiliadis medium (MSRV). For testing, the lateral flow device is inserted into the MSRV culture following incubation.

For testing of raw shell eggs, chicken carcass rinse, and poultry feed, product was inoculated with SE and held under conditions expected to produce inoculum stress. Poultry house environmental samples were tested without inoculation. Test portions were analyzed using the Reveal 2.0 method and the appropriate reference culture procedure (FDA/BAM, USDA/MLG, or NPIP, depending on the sample type). For each type of product that was inoculated with SE, 20 fractional-level (25–75% positive), five high-level, and five uninoculated control portions were prepared and tested.

Results showed that the Reveal 2.0 method was as effective as the reference culture methods for detection of SE in all sample types studied, as determined by chi-square or probability of detection analysis. It is concluded that the Reveal 2.0 method is an effective procedure for detection of SE in a variety of egg and poultry sample types, while providing results within 48 hours.

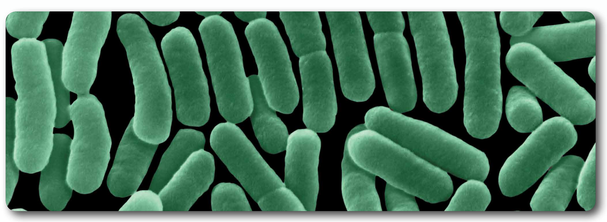
INTRODUCTION

Reveal 2.0 for *Salmonella* Enteritidis is a second-generation lateral flow immunoassay diagnostic product for detection of SE in a variety of poultry-associated matrices. It replaces the original Reveal for SE device, which achieved PTM status as method #111001 [1].

The most notable features of the new test are: 1) A new device format as a "naked strip", (i.e., without a housing), to be run in a vertical orientation; and 2) Use of a novel 2-step enrichment protocol employing tetrathionate and MSRV media. This combination has been shown to be especially productive in recovery of SE from poultry-associated matrices [2].

In inclusivity testing, the new Reveal device detected all 53 strains of SE examined. The device also detected 17 of 18 serovars of non-SE group D1 *Salmonella* spp. tested, producing a negative result only with *S.* Gallinarum. The device produced negative results with 24 strains of non-group D1 salmonellae and other members of the *Enterobacteriaceae* (Neogen Corp., unpublished results).

Here we describe results of validation studies for a variety of matrices associated with poultry and egg production and processing.



MATERIALS AND METHODS

INOCULUM STRAINS

Raw shell eggs, chicken carcass rinse, 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 iodine/potassium iodide mixture in accordance with the FDA/BAM reference method [3]. Eggs were cracked and a pool of 1,400 eggs prepared. 10 L was withdrawn to serve as uninoculated control material. Another 10 L was withdrawn to inoculate at a high level. Additional material was reserved for MPN analysis. The remaining eggs were reserved for the fractional level inoculation. Eggs were inoculated with a dilution of an overnight culture at the following levels: High – 15 cfu/20 eggs, fractional – 0.75 cfu/20 eggs. Inoculated and control eggs were divided into 20-egg portions (1 L each) and held at 2–8°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±2°C for 24±2 hours. Following the primary incubation, 0.1 mL of the culture was transferred to a tube containing 2.5 mL MSRV, and incubated at 42±0.2°C for 24±2 hours.

For the FDA/BAM reference method, test portions held at 2–8°C were incubated at room temperature for 96 hours. Analysis was continued in accordance with the published method [3].

CHICKEN CARCASS RINSE

Thirty chickens were obtained. Five were reserved for use as uninoculated controls, five for inoculation at a high level, and 20 for inoculation at a fractional level. The inoculum was applied as 1 mL of culture dilution in the bird cavity. Spike levels were: High – 150 cfu/bird, fractional – 75 cfu/bird. Inoculated birds were held at 2–8°C for 48–72 hours. After the hold, 400 mL buffered peptone water (BPW) was added to the cavity, the carcasses were shaken for 1 minute, and two 30 mL portions of rinse removed for analysis.

For the Reveal method, 30 mL of Reveal 2.0 for SE Medium with 5 mg/L novobiocin was added and the culture incubated for 24 hours at 35±2°C. Secondary enrichment in MSRV was performed as described above.

The reference method used for this sample type was that of USDA/MLG [4]. 30 mL BPW was added to the rinse sample and the analysis continued in accordance with the published method.

POULTRY FEED

A lyophilized cell pellet of SE was prepared, ground in a mortar and pestle, and used to inoculate poultry feed. After extensive mixing, the inoculated material was allowed to stabilize at room temperature for 14 days. An MPN analysis was performed to determine the level of surviving salmonellae, then inoculated material was blended with additional feed to produce test portions at the desired levels of inoculation. Ten portions were prepared at a high level, 40 at a fractional level, and 10 as controls. Half of the test portions were tested using the Reveal method and the other half using the FDA/BAM reference culture procedure [3].

For the Reveal method, a 25 g sample was combined with 225 mL Reveal 2.0 for SE Medium with 5 mg/L novobiocin and incubated at 35±2°C for 24±2 hours. Secondary enrichment in MSRV was performed as described above.

For the FDA/BAM reference method, 25 g sample was combined with 225 mL lactose broth, and the analysis continued in accordance with the published method.

POULTRY HOUSE ENVIRONMENTAL SAMPLES

Environmental samples such as drag swabs, chick papers, and fluff were enriched in 100 mL tetrathionate Hajna broth for 24±2 h at 35±2°C.

For the Reveal method, secondary enrichment in MSRV was performed as described above.

The reference method for this comparison was that of the National Poultry Improvement Plan [2]. The same tetrathionate culture used for the Reveal method served as the primary enrichment, followed by plating to MSRV agar, and analyzing a sample from the leading edge of growth for the presence of SE.

REVEAL DEVICE TESTING

To perform the Reveal test, the test strip is inserted into the tube containing the incubated MSRV secondary enrichment culture. The strip is allowed to develop for 10 minutes at room temperature, then examined for the presence of visible test and control lines.

For culture confirmation of Reveal results, a loopful of the MSRV culture is streaked to *Salmonella* selective/differential agars, and identification of presumptive isolates continued using standard biochemical and serological procedures [3, 4].

RESULTS

Results for raw shell eggs, chicken carcass rinse, and poultry feed are shown in **Table 1**. There were no significant differences in the number of positive results by the Reveal and reference methods for any of the commodities tested as determined by chi-square analysis ($P < 0.05$) [5]. 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 MSRV secondary enrichment.

Results of poultry house environmental sample testing from third-party laboratories are shown in **Table 2**. There were a total of 638 samples tested in a paired design (common primary enrichment). There were 85 positives by the Reveal method, of which 64 were confirmed independently from the MSRV tube, vs. 62 by the NPIP reference method. Confirmation of SE was especially difficult in laboratory 3, for both the Reveal and NPIP reference methods, as these samples were heavily co-contaminated with group E4 *Salmonella* spp. The use of anti-SE immunomagnetic beads aided 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 were no Reveal-negative, NPIP reference method-positive results.

TABLE 1. RESULTS OF TESTING OF RAW SHELL EGGS, CHICKEN CARCASS RINSE, AND POULTRY FEED

Matrix	Inoc. Strain	Level (cfu/portion)[a]	N	Reveal[b]	Reveal Confirmed[c]	Reference Method[d]	x^{2e}
Raw shell eggs	SE USDA 97-15777	–	5	0	0	0	–
		< 3.0	20	9	9	13	1.58
		43	5	5	5	5	–
Chicken carcass rinse	SE GPL 10420847	–	5	0	0	0	–
		2.8	20	16	15	15	0.00
		–	5	5	5	5	–
Poultry feed	SE 1279 (M. Saeed)	–	5	0	0	0	–
		1.1	20	15	15	9	3.66
		1.9	5	4	4	3	–

[a] Determined by MPN analysis.
[b] Number of test portions positive by Reveal assay not considering subsequent culture confirmation.
[c] Number of test portions positive by Reveal assay and confirmed by culture from associated enrichments.
[d] Number of test portions positive by FDA/BAM (shell eggs, poultry feed) or USDA/MLG (carcass rinse) reference method.
[e] $x^2 > 3.84$ indicates a significant difference at $P < 0.05$.

TABLE 2. RESULTS OF ENVIRONMENTAL SAMPLE TESTING

LABORATORY 1

		NPIP Reference +	NPIP Reference –	NPIP Reference Total
Reveal	+	19	5[a]	24
	–	0	362	362
	Total	19	367	386

[a] Five positive results could not be confirmed from MSRV tube.

LABORATORY 2

		NPIP Reference +	NPIP Reference –	NPIP Reference Total
Reveal	+	16[b]	13[c]	29
	–	0	82	82
	Total	16	95	111

[b] Six positive results could not be confirmed from MSRV tube.
[c] Seven positive results could not be confirmed from MSRV tube.

LABORATORY 3

		NPIP Reference +	NPIP Reference –	NPIP Reference Total
Reveal	+	27	5[d]	32
	–	0	109	109
	Total	27	114	141

[d] Three positive results could not be confirmed from MSRV tube.

TOTAL

		NPIP Reference +	NPIP Reference –	NPIP Reference Total
Reveal	+	62[e]	23[f]	85
	–	0	553	553
	Total	62	576	638

[e] Six positive results could not be confirmed from MSRV tube.
[f] 15 positive results could not be confirmed from MSRV tube.

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

[1] Jagadeesan, B., Curry, S., Foti, D., Peterson, L., Wilson, R., & Mozola, M. (2011) Reveal *Salmonella* Enteritidis test for detection of *Salmonella* Enteritidis in shell eggs and environmental samples. *J. AOAC Intl.* 94, 1125-1137.

[2] Waltman, D. (2012) *Salmonella* isolation and detection methods http://www.aphis.usda.gov/animal_health/animal_dis_spec/poultry/downloads/presentations/2012_presentations/salmonella_presentation.pdf

[3] US FDA (2011) *Bacteriological Analytical Manual*, chapter 5 http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm070149.htm

[4] USDA-FSIS (2011) *Microbiology Laboratory Guidebook*, chapter 4.05 http://www.fsis.usda.gov/PDF/MLG_4_05.pdf

[5] Mantel, N. & Haenszel, W. (1959) *J. Nat. Cancer Inst.* 22, 719-748

ACKNOWLEDGEMENTS

We thank Dr. Doug Waltman of the Georgia Poultry Laboratory and Gordon Whitbeck of Whitbeck Laboratories for performing some of the environmental sample analysis. We also thank T. Frana, D. Waltman, and M. Saeed for providing SE strains.