Evaluation of a Real-Time PCR Method to Detect *Salmonella* Enteritidis in Whole Shell Eggs and Environmental Poultry Samples

Robert S. Tebbs[1], Peyman Fatemi[1], Olga V. Petrauskene[1],Arlene Nunez[1], Craig A. Cummings[1], Erin Crowley[2], Patrick Bird[2], Kiel Fisher[2], James Agin[2], Pius M. Brzoska[1], David Goins[2], Manohar R. Furtado[1], and Cate O'Connell[1].

[1]Life Technologies/Applied Biosystems, Foster City, CA, 94404, [2]Q Laboratories, Cincinnati, OH,

BACKGROUND

Salmonella enterica serovar Enteritidis (*Salmonella* Enteritidis, or SE) together with *Salmonella* Typhimurium, account for nearly half of all *Salmonella* illnesses in the US. The CDC estimates that 75% of SE outbreaks are associated with the consumption of raw or poorly cooked shell eggs. Beginning in July of 2010 the U.S. Food and Drug Administration mandated routine environmental testing of poultry houses for presence of *Salmonella* Enteritidis (1). If SE is detected in the environment then eggs from those houses must be tested prior to their distribution for sale.

PURPOSE

To develop and validate a rapid real-time PCR system for detecting *Salmonella* Enteritidis in whole shell eggs and environmental drag swabs. The complete system will include a shortened enrichment method automated sample preparation, and custom software for data analysis.

METHODS

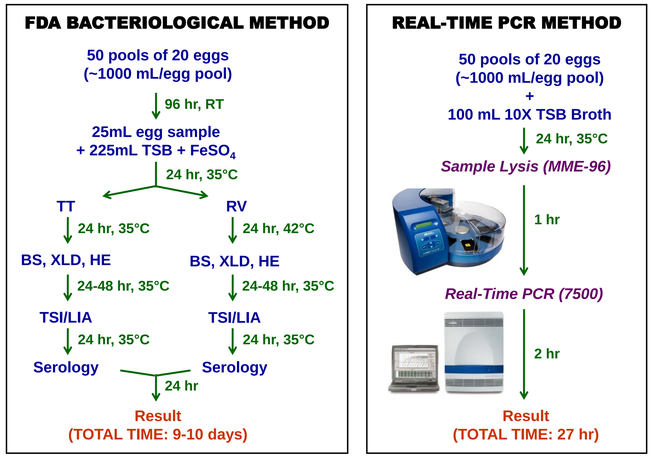
Egg Sample Preparation and Enrichment
Whole shell eggs were disinfected with alcohol:iodine. Approximately 250 eggs were combined into a bulk sample for the uninoculated control, and a bulk lot consisting of approximately 900 eggs were inoculated with SE (ATCC 13706) at a concentration of 0.2-2 CFU per 1000 g. Egg pools consisting of approximately 20 eggs (1000 g) were prepared. Twenty inoculated and 5 uninoculated egg pools were enriched according to the FDA BAM Chapter 5 protocol for *Salmonella* in egg samples. A second set of 20 inoculated and 5 uninoculated egg pools were combined with 100 mL of 10X TSB per pool, and then incubated at 35°C for 24 hours.

Poultry Drag Swab Sample Preparation and Enrichment
Poultry drag swabs were collected from a low incidence poultry house and shipped overnight. Drag swabs making up one experimental set consisted of twenty (20) replicates artificially contaminated with *S.* Enteritidis and five (5) un-inoculated control swabs. The drag swabs were individually inoculated with 0.5 mL of inoculum and allowed to equilibrate for 48 hours at 4 ±2°C. One set of drag swabs were assayed by the FDA-BAM reference method. A second set of drag swabs were enriched with 100 mL of Tetrathionate broth containing 2.0 mL of iodine-iodide solution and 1.0 mL 0.1% Brilliant Green solution and then assayed using the TaqMAN SE method and confirmed culturally by the NPIP MSRV method.

DNA Extraction and Real-Time PCR
Sample preparation used the PrepSEQ® NA Extraction Kit automated on the MagMAX™ Express-96 Magetic Particle Processor. Real-time was run on the 7500 Fast instrument using standard conditions (95 ºC for 10 min; 40 cycles at 95 ºC for 15 seconds and 60 ºC for 60 seconds).

Statistical Analysis
Results obtained from the TaqMan® method were compared to those from the FDA BAM reference methods (2,3) using the Mantel-Haenzel chi-square analysis for unmatched test portions. Statistical methods were as described by the AOAC International Methods Committee (4).

Figure 1. Sample Processing: FDA Method vs. Real-Time PCR



RESULTS

Figure 2. FDA BAM vs. Real-Time PCR for Whole Shell Eggs

Experiment 1			
		Lysis	
Sample Name	FDA BAM confirmed	FAM (SE)	VIC (IPC)
1	-	ND	30.0
2	-	ND	29.9
3	+	20.3	29.0
4	+	22.9	28.7
5	-	ND	29.9
6	+	21.1	29.2
7	+	22.0	29.1
8	-	ND	34.7
9	+	19.1	30.1
10	+	18.4	30.8
11	+	19.2	29.4
12	+	20.2	29.3
13	+	18.6	29.7
14	+	18.5	29.5
15	-	ND	30.6
16	+	21.3	30.2
17	-	ND	30.1
18	-	ND	30.1
19	-	ND	30.3
20	-	ND	30.1
21	+	17.2	32.2
22	+	19.6	29.1
23	+	18.1	29.9
24	+	23.5	32.6
25	+	21.0	28.9
TOTAL +	16	16	N/A

Experiment 2			
		Lysis	
Sample Name	FDA BAM confirmed	FAM (SE)	VIC (IPC)
1	-	ND	31.0
2	+	26.2	29.9
3	-	ND	31.1
4	+	27.0	29.6
5	-	ND	32.0
6	+	27.9	30.8
7	+	25.1	29.8
8	-	ND	31.7
9	-	ND	31.6
10	+	26.2	30.2
11	+	25.1	30.0
12	-	ND	31.5
13	+	28.5	30.9
14	+	23.2	29.4
15	-	ND	32.1
16	+	25.7	30.0
17	-	ND	31.7
18	+	24.8	29.8
19	-	ND	31.7
20	+	23.6	29.3
21	-	ND	30.7
22	+	27.1	30.0
23	-	ND	31.1
24	-	ND	31.3
25	+	22.5	29.3
TOTAL +	13	13	N/A

All egg pool samples tested by the TaqMan® SE Real-time PCR method were correctly called when confirmed by the FDA BAM method. The Ct values in the low to mid 20's is an indication of excellent enrichment.

Figure 3. FDA BAM vs. Real-Time PCR for Environmental Drag Swabs

Experiment 1			
	MSRV	Lysis	
Sample Name	NPIP confirmed	FAM (SE)	VIC (IPC)
1	+	20.8	27.7
2	-	ND	31.8
3	+	17.5	28.1
4	+	31.4	30.9
5	-	ND	30.5
6	-	ND	31.2
7	+	18.7	27.9
8	-	ND	29.7
9	+	31.1	29.5
10	-	ND	29.9
11	+	17.8	29.4
12	+	18.0	28.5
13	+	31.4	30.3
14	-	ND	29.6
15	-	ND	30.7
16	-	ND	30.4
17	+	23.3	29.1
18	-	ND	32.4
19	-	ND	30.8
20	-	ND	32.1
21	+	17.2	29.7
22	+	23.0	28.6
23	-	ND	30.8
24	-	ND	32.5
25	+	35.2	30.7
TOTAL +	12	12	N/A

Experiment 2			
	MSRV	Lysis	
Sample Name	NPIP confirmed	FAM (SE)	VIC (IPC)
1	-	ND	29.8
2	+	28.2	29.8
3	-	ND	31.1
4	-	ND	29.6
5	-	ND	32.1
6	+	33.1	29.8
7	-	ND	29.7
8	-	ND	30.3
9	-	ND	31.0
10	+	33.4	29.8
11	+	29.1	29.4
12	+	32.8	29.7
13	+	33.4	29.3
14	+	32.9	29.5
15	-	ND	31.5
16	+	29.2	29.7
17	-	ND	30.4
18	-	ND	30.6
19	-	ND	29.9
20	-	ND	30.7
21	-	ND	29.6
22	-	ND	30.1
23	-	ND	31.1
24	-	ND	31.8
25	+	28.2	30.2
TOTAL +	9	9	N/A

All drag swab samples tested by the TaqMan® SE Real-time PCR method were correctly called when confirmed by the MSRV NPIP method.

Table 1. Summary Data Table for Whole Shell Eggs

Inoculation Level	Inoculating Organism	U.S. FDA BAM	TaqMan® Salmonella Enteritidis Method		X^2	Relative Sensitivity	False Negative Rate	False Positive Rate
			Presumed	Confirmed				
Experiment 1								
Control	N/A	0/5	0/5	0/5	-	-	0%	0%
Spike	*S. enterica* ser. Enteritidis ATCC 13076	16/20	16/20	16/20	0	100%	0%	0%
Experiment 2								
Control	N/A	0/5	0/5	0/5	-	-	0%	0%
Spike	*S. enterica* ser. Enteritidis ATCC 13076	11/20	13/20	13/20	0.41	118%	0%	0%

Table 2. Summary Data Table for Poultry House Drag Swabs

Inoculation Level	Inoculating Organism	U.S. FDA BAM	TaqMan® Salmonella Enteritidis Method		X^2	Relative Sensitivity	False Negative Rate	False Positive Rate
			Presumed	Confirmed				
Experiment 1								
Control	*N/A*	*0/5*	*0/5*	*0/5*	-	-	*0%*	*0%*
Spike	*S. enterica ser. Enteritidis ARS-12*	*9/20*	*12/20*	*12/20*	*0.88*	*133%*	*0%*	*0%*
Experiment 2								
Control	*N/A*	*0/5*	*0/5*	*0/5*	-	-	*0%*	*0%*
Spike	*S. enterica ser. Enteritidis ATCC 13076*	*7/20*	*9/20*	*9/20*	*0.41*	*128%*	*0%*	*0%*

The results from chi-square analysis on two independent experiments indicated no difference between the FDA BAM reference method and the TaqMan® Real-time PCR method.

SUMMARY

The FDA has determined that the workflow using the TaqMan® Salmonella Enteritidis Detection Kit is equivalent in accuracy, precision, and detection sensitivity to the test method set forth in the Chapter 5 (Salmonella) of the FDA's Bacteriological Analytical Manual (BAM, December 2007 Edition) for whole shell eggs, and to the "Environmental Sampling and Detection of Salmonella in Poultry Houses" (2008) for environmental drag swabs. In addition, the NPIP has granted interim approval for the TaqMan® Salmonella Enteritidis Detection Kit workflow as a rapid screening method for Salmonella Enteritidis in environmental drag swab samples.

CONCLUSIONS

- The TaqMan® Real-time PCR workflow shows equivalence in performance to the FDA BAM protocol for detecting SE in whole shell eggs and environmental drag swabs.
- The TaqMan® Real-time PCR SE assay showed no false positive and no false negative detection of SE in multiple experiments in either eggs or drag swabs.
- The TaqMan® Real-time PCR SE assay allows detection of SE in approximately 27 hrs (~3 hr post enrichment).
- The TaqMan® Real-time PCR SE workflow was designed to be simple and sample preparation is fully automated.
- The method detected all SE strains tested (N=37), and demonstrated no detection of non-SE strains (N=62) (results not shown).

REFERENCES

1. Prevention of Salmonella Enteriditis in Shell Eggs During Production, Storage, and Transportation; Final Rule (July 9, 2009). Federal Register, Vol 74 (130): 33030-33101.
2. US FDA Bacteriological Analytical Manual Online, Chapter 5, Salmonella, February 2007 version, http://www.fda.gov/downloads/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/UCM244774.pdf. Accessed on July 16, 2012.
3. US FDA Environmental Sampling and Detection of Salmonella in Poultry Houses, October 2008 version, http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ucm114716.htm. Accessed on July 16, 2012.
4. AOAC Interational Qualitative and Quantitative Microbiology Guidelines for Methods Validation 9). J. AOAC International, Vol (2):402-416

For research use only. Not intended for any animal or human therapeutic or diagnostic use.