

Reduced enrichment time and threshold testing for *Salmonella* spp.

BIO-RAD

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Introduction

Regulators and food producers are currently encountering hurdles to further reduce the incidence of foodborne *Salmonella* infections. Increasing importance has been placed on quantification methods and their ability to reduce risk. When testing raw meat and poultry with limited shelf life, food producers need fast results to make enhanced food safety and processing decisions. This study demonstrates methods to reduce enrichment times for the detection of *Salmonella* spp. along with the ability to estimate original food matrix contamination levels.

Methods

Limit of Detection (LoD) Protocol

Salmonella cultures (Table 1) were incubated overnight followed by an overnight cold adaptation. Strains were either combined into a cocktail containing roughly the same CFU/ml of each strain or tested individually. Sixty-five-gram 93% lean ground turkey, ground beef, or beef trim samples were inoculated and held overnight before enrichment. Samples were inoculated at approximately ≥ 1 (LoD 1) or ≥ 10 (LoD 10) CFU/g levels (note this is not CFU total in a sample but rather CFU per gram of food matrix). A separate set of five samples for each time point were used with standard incubators (at Bio-Rad) or one set of 5 samples when using a walk-in incubator (at Food Safety Net Services) to minimize the influence of temperature fluctuations. Samples were enriched using double strength Buffered Peptone Water Plus (BPW Plus, Bio-Rad Laboratories, catalog #3564684) added at a 1:3 ratio (65 g ground turkey and 195 ml 2x BPW prewarmed to 48°C) and incubated at 42°C. Real-time PCR analysis was performed using the iQ-Check *Salmonella* II kit (Bio-Rad Laboratories, catalog #3578123), Easy II extraction, and "SalmoFast" APF. Three aliquots from each bag were tested at each time point to measure the reproducibility of the results. Colonies were confirmed by either real-time PCR or *Salmonella* Confirm Latex/Kit (Bio-Rad Laboratories, catalog #3556711).

Test Matrices

- 93% lean ground turkey tested at Bio-Rad Laboratories Industrial Applications Lab
- Ground beef and beef trim tested at Food Safety Net Services (FSNS)

Test Strains

Table 1. Strains used to prepare cold adapted cocktail inoculum or tested individually. RDC indicates internal Bio-Rad strain collection.

<i>Salmonella</i> enterica subsp. enterica Serotype	Reference Number	Cocktail 1	Cocktail 2	Individual
Enteritidis	ATCC 13076	X		
Heidelberg	RDC 93	X		
Kentucky	RDC 1095	X		
Newport	RDC 92	X		
Typhimurium	ATCC 14028	X	X	X
Kentucky	ATCC 9263		X	
Newport	ATCC 6962		X	
Anatum	ATCC 9270		X	
Montevideo	ATCC 8387		X	X
Dublin	ATCC 15480			X

Results

Results demonstrated the detection of *Salmonella* in 3-4 hr for LoD 10 and 4-5 hr for LoD 1 (depending on the food matrix). Ground turkey samples were 100% positive at the LoD 1 level after 5 hr incubation and 100% at the LoD 10 level after 4 hr incubation (Table 2, Fig. 1). Independent testing at FSNS demonstrated shorter incubation times for beef samples at 3 hr for LoD 10 and 4 hr for LoD 1 (Tables 3 & 4). Small variations in laboratory equipment, enrichment media, processing plant interventions, ingredients, and other matrix effects can influence shortened enrichment performance. These data from two laboratories and three food matrices also demonstrate the need for laboratories to verify LoD incubation time points using the intended food matrices and their laboratory equipment.

Table 2. Data produced at the Bio-Rad Hercules Industrial Applications Lab comparing detection of *Salmonella* at various time points and inoculation levels.

93% Lean Ground Turkey		% Positive Aliquots			
Inoculum	Inoculated CFU/g	2 hr	3 hr	4 hr	5 hr
Cocktail 1	2.7	-	-	73%	100%
Cocktail 1	2.3	-	-	53%	100%
Cocktail 1	15.6	-	33%	100%	-

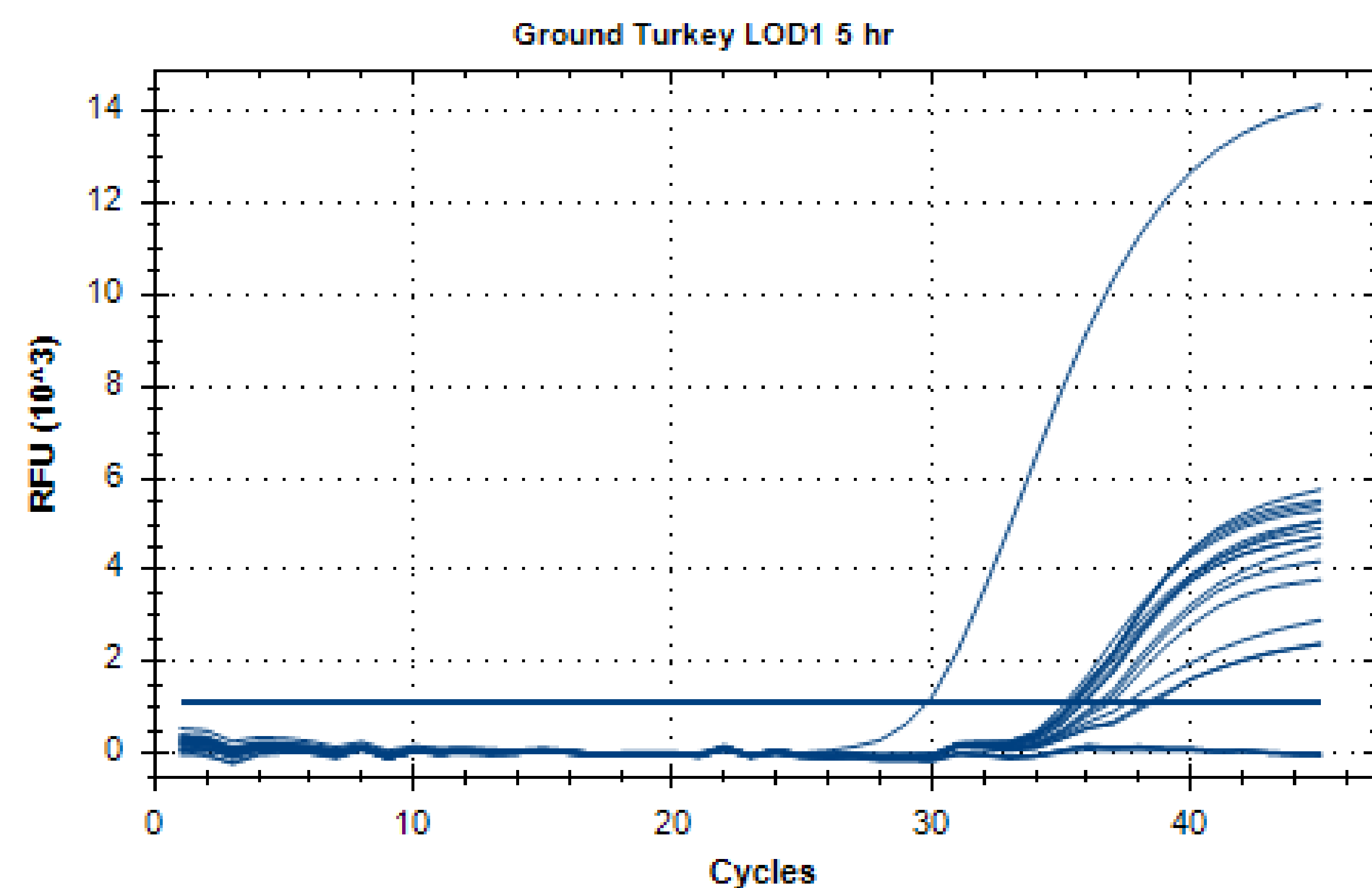


Fig 1. Example of real-time PCR curves when testing ground turkey at 5 hr for *Salmonella* spp. at LOD1 threshold.

Results

PCR results for *L. monocytogenes* (Table 1) and *Salmonella* (Table 2) test method samples were identical to culture confirmation. When compared to the reference methods, the POD was found to be identical for the *Salmonella* test method and statistically equivalent for the *L. monocytogenes* test method.

Table 3. Independent data produced by Food Safety Net Services comparing detection of *Salmonella* in ground beef at various inoculation levels and time points.

Ground Beef		% Positive Aliquots			
Inoculum	Inoculated CFU/g	2 hr	3 hr	4 hr	5 hr
Typhimurium	0.32	-	20%	47%	100%
Dublin	0.30	-	20%	93%	100%
Montevideo	0.42	-	7%	87%	100%
Cocktail 2	0.37	-	13%	100%	100%
Typhimurium	7.20	13%	40%	100%	100%
Dublin	6.80	47%	73%	100%	100%
Montevideo	10.90	40%	100%	100%	100%
Cocktail 2	20.70	80%	100%	100%	100%

Table 4. Independent data produced by Food Safety Net Services comparing detection of *Salmonella* in beef trim at various inoculation levels and time points.

Beef Trim		% Positive Aliquots			
Inoculum	Inoculated CFU/g	2 hr	3 hr	4 hr	5 hr
Typhimurium	0.64	-	47%	87%	100%
Dublin	0.76	-	93%	100%	100%
Montevideo	0.84	-	67%	100%	100%
Cocktail 2	1.10	-	80%	100%	100%
Typhimurium	14.00	33%	93%	100%	100%
Dublin	13.25	27%	100%	100%	100%
Montevideo	12.94	47%	100%	100%	100%
Cocktail 2	12.02	67%	100%	100%	100%

Significance

These data demonstrate the ability to reduce the incubation time necessary for the detection of *Salmonella* spp. (Table 5). This also shows that incubation times can be combined with the test method limit of detection to estimate original contamination levels of a food matrix. These data also demonstrate the need for laboratories to verify or tailor LoD incubation time points using the intended food matrices and their laboratory equipment.

Table 5. Summary of conditions necessary for the detection of *Salmonella* spp.

Condition	Parameter
Media	2x BPW
Matrix to Media Ratio	1:3
Media Preheating Temperature	48°C
Incubation Temperature	42°C
LoD1 (1 CFU/g) Incubation Period	5 hr (ground turkey) 4 hr (beef)
LoD10 (10 CFU/g) Incubation Period	4 hr (ground turkey) 3 hr (beef)
iQ-Check <i>Salmonella</i> II Extraction	Easy II
RT-PCR Program (APF)	Salmo Fast

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