

# Evaluation of Loop-Mediated Isothermal Amplification for the Detection of *Salmonella* from Poultry Matrices

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## Abstract

Loop-mediated isothermal amplification (LAMP) has emerged as an alternative to PCR based methods for detection of food-borne pathogens, offering simple, easy to use, detection technology with high speed, efficiency, sensitivity and specificity. The performance of LAMP-bioluminescent assay as an alternative method for the detection of *Salmonella* in primary production samples, poultry rinses and raw poultry products compared to PCR was evaluated. After enrichment, boot swabs from poultry farms, carcass rinses and raw poultry products were tested by a LAMP-bioluminescent and a PCR assay. The LAMP-bioluminescent assay was able to detect *Salmonella* in the various matrices tested and had higher or equivalent sensitivity and specificity to the PCR method used. No significant difference (95% confidence interval) was found between the LAMP and PCR method as determined by probability of detection analysis. The *Salmonella* LAMP-bioluminescent assay enabled reliable and rapid detection of *Salmonella* in variety of poultry matrices and is an acceptable alternative to the PCR method.

## Keywords

*Salmonella*, isothermal amplification, LAMP, poultry, PCR

## 1. Introduction

*Salmonella* is globally recognized as a major cause of foodborne infection in humans. About 1.35 million illnesses occur each year in the US due to salmonellosis and food is the source of most of these illnesses [1] resulting in significant economic burden [2, 3]. *Salmonella* is a significant problem in the poultry industry throughout the world [4, 5]. Raw poultry products are contaminated with *Salmonella* and *Campylobacter* due to rupturing of contaminated gut contents (small intestine and ceca) during slaughter operations [4, 6]. In addition, contamination of bird's feathers and skin in the farm can bring these bacteria to the processing facilities [4]. Proper sanitary operations and use of antimicrobial interventions are necessary to minimize the contamination during slaughter and processing of the carcasses into parts and comminuted products. Government agencies, such as the US Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), have implemented pathogen reduction performance standards for *Salmonella* and *Campylobacter* in raw chicken parts, ground chicken and ground turkey [7, 8]. Performance standards are used by the USDA FSIS to assess establishment performance for various poultry products. Recently, USDA announced plans to mobilize a stronger and more comprehensive effort to reduce *Salmonella* illnesses associated with poultry products with a goal to achieve the national target of a 25% reduction in *Salmonella* illnesses [9]. These efforts have necessitated continued and increased surveillance of *Salmonella* in the poultry industry. While the advances in rapid methods such as immunoas-

says and PCR have enabled accurate detection of foodborne pathogens [10-14], there is still a need for a quicker, simpler and less expensive technology.

Loop-mediated isothermal amplification (LAMP) can amplify DNA under isothermal conditions (60 to 65°C) with high specificity and sensitivity in 60 min or less [15-18]. The DNA amplification is driven by *Bst* polymerase, a unique enzyme with DNA strand-displacement activity that enables the continuous, rapid isothermal amplification of DNA. LAMP uses multiple primers to recognize distinct regions of the genome and *Bst* DNA polymerase to provide continuous and rapid amplification of genetic material [15-18]. Compared to PCR, LAMP performs better as it has greater ability to amplify DNA in the presence of interfering substances often found in clinical, food and environmental samples [19-24]. LAMP has been coupled to a bioluminescent assay for the detection of amplified products, enabling simultaneous detection and amplification which provides real-time results and a short run time [25]. The LAMP-bioluminescent method offers a simpler, faster and streamlined approach to pathogen detection [26-33]. The *Salmonella* LAMP-bioluminescent assay, 3M™ Molecular Detection Assay 2 - *Salmonella* (MDA2SAL96) has been used for the detection of *Salmonella* in a variety of food matrices [26-33] and is equivalent to standard culture methods.

The objective of this study was to evaluate the performance of a *Salmonella* LAMP-bioluminescent assay for the detection of *Salmonella* in poultry rinses and raw poultry products as compared to a PCR assay. *Salmonella* LAMP assay was also evaluated for detection of *Salmonella* in primary production samples (boot swabs) as compared to the US National Poultry Improvement Plan (NPIP) method [34].

## 2. Materials and methods

The enrichment conditions for both the LAMP (MDA2SAL96, 3M Food Safety, St. Paul, MN) and the PCR method (BAX® System PCR assay for *Salmonella*, Hygiena LLC, Camarillo, CA) for various matrices are summarized in Table 1. The samples were enriched per recommended protocol (Table 1) and analyzed by the LAMP method (MDA2SAL96) or the PCR method (BAX® System PCR assay for *Salmonella*). For all samples analyzed with LAMP-bioluminescent assay, randomly selected samples were also analyzed with the external amplification control, 3M™ Molecular Detection Matrix Control (3M Food Safety), to assess sample interference during the amplification reaction. All samples analyzed by both molecular method were culture confirmed as outlined in Figures 1-3. Total aerobic counts in all matrices were also determined by serially diluting the sample homogenates in Butterfield's phosphate buffer (3M Food Safety) and plating on 3M™ Petrifilm™ Aerobic Count Plates (3M Food Safety). The plates were incubated for 48 hours at 35 ± 1°C and colonies were enumerated.

**Table 1. Sample preparation and enrichment protocols for *Salmonella* detection**

Sample matrix	Sample size	Enrichment medium	Enrichment temperature (± 1°C)	Enrichment time (hours)	Enrichment medium		
					MDA2SAL96		BAX® System PCR assay for <i>Salmonella</i>
Whole bird carcass or parts rinsed in 400 mL of BPW	30 mL of rinsate	30 mL BPW ISO	41.5	18-24	30 mL BPW	35	20-24
Raw poultry products	325 g	975 mL (pre-warmed) BPW ISO	41.5	14-24	1,625 mL BPW	35	20-24
NPIP method Boot swabs from primary production	1 boot swab	100 mL TT broth	37	20-24			

### 2.1. Preparation of *Salmonella* inoculum

Three *Salmonella* isolates were used in this study, *Salmonella enterica* Heidelberg (ATCC 8326), *Salmonella enterica* Senftenberg (ATCC 43845), and wild isolate *Salmonella enterica* Enteritidis. The ATCC strains were obtained from ATCC, Manassas, VA and the wild isolate was from the culture collection of WBA Analytical Laboratories, Springdale, AR. To prepare a cocktail of *Salmonella* inocula, an isolated colony from tryptic soy agar (TSA, Edge Biologicals, Memphis, TN) was inoculated into 10 mL of Brain Heart Infusion Broth (BHI, Becton Dickinson, Franklin Lakes, NJ) using a sterile inoculating loop and incubated for 18 hours at 35°C. After incubation, all three BHI tubes were combined in one sterile 50 ml centrifuge tube and centrifuged at 5000 x g for 15 min at 6°C. The pellets were washed twice with 30 mL of sterile 0.85% saline and resuspended in 0.85% saline. Serial 10-fold dilutions of suspensions were prepared in 0.85% saline and 100 microliter of the dilutions was spread plated on TSA and incubated at 35°C for 18 hours. The

colonies on TSA plates were counted, and an average count of each dilution was used to determine appropriate amount of inoculum added to each sample.

## 2.2. Primary production samples

### 2.2.1 Boot swabs

Thirty samples of boot swabs were collected in two farms ( $n=30/\text{farm}$  for a total of 60 samples) for *Salmonella* testing using sterile boot swab (cotton-poly blend fabric sock style boot cover) pre-moistened with double-strength skim milk (Solar Biologics Inc., Massena, NY). Briefly, a technician placed disposable plastic boot cover over the shoes to protect personal clothing and prevent cross contamination of the sample from shoe sole carryover. Then technician removed a sterile pre-moistened boot swab from the twirl-tie bag and placed over the boot cover and walked the entire length of one side of the poultry house. After sample collection, the boot swab was removed, immediately transferred to the original twirl-tie bag and transported to the the laboratory for further processing. One hundred mL of sterile TT broth (Edge Biologicals) was added to each of the bags. All the boot swabs were incubated at 37°C for 22 hours. The flow chart for detection of *Salmonella* in boot swabs is shown in Figure 1.

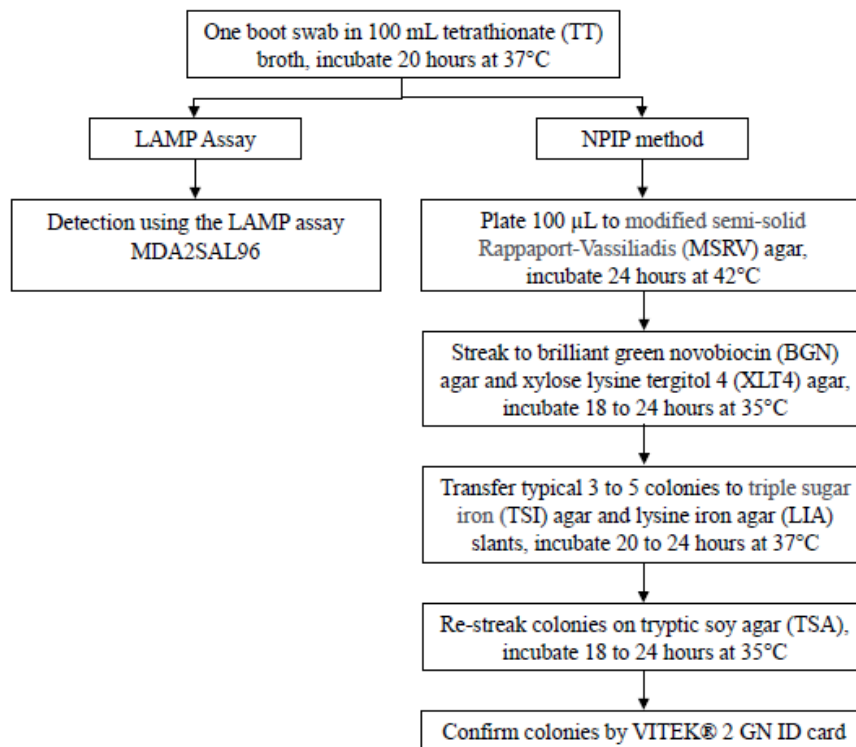


Figure 1. Flow chart for detection of *Salmonella* in primary production boot swabs by MDA2SAL96 and NPIP method.

## 2.3. Poultry sample collection

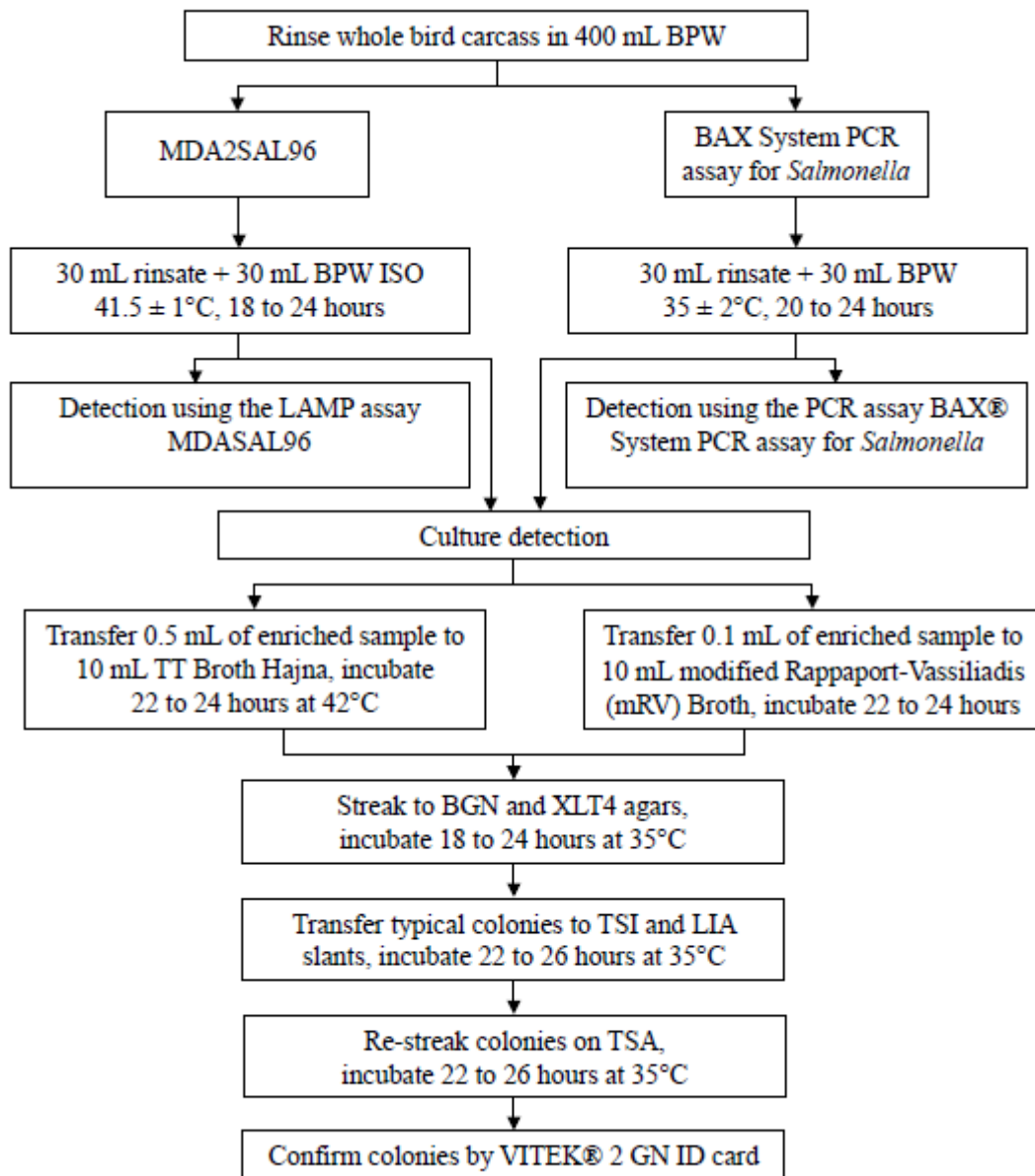
Whole chicken carcass rinsates and raw poultry products were collected from a commercial broiler abattoir. The samples were collected from the processing line at three sampling points (rehang, post-chill intervention and poultry parts processing).

### 2.3.1 Rinsate collection

Carcasses were collected from the poultry processing line, allowed to drain for at least 1 minute and aseptically transferred into a sterile poultry rinse bag (Nasco, Fort Atkinson, WI). A total of 400 mL of cold (kept at 2-8°C) buffered peptone water (BPW) (3M Food Safety) was added to each bag by pouring approximately half of the solution into the interior cavity and half onto the exterior of the carcass. The bag was closed to prevent leakage and the carcass or parts were rinsed by gently shaking for 1 minute using a 1-foot arm-arcing motion. The rinsate was aseptically transferred back into the original container, capped tightly and labeled with sample information. Rinsates were then transported in a cooler with ice to the laboratory and used for further testing.

### 2.3.2 Rinsate testing

BPW rinsates were evaluated in an unpaired study to compare the LAMP method for detection of *Salmonella* against the PCR method. The flow chart for detection of *Salmonella* in rehang and post-chill rinsates is shown in Figure 2. A total of 120 rinsates, 30 rinsates from rehang carcasses and 30 rinsates from post-chill carcasses for each method were analyzed for *Salmonella* detection (total of 60 rinsates for each method).



**Figure 2. Flow chart for detection of *Salmonella* in poultry carcass or parts rinse by MDA2SAL96 and PCR method and culture confirmation.**

For each of the method, 30 mL of the rinsate was added to a sterile Samco™ Narrow Mouth Bio-Tite™ 90 mL specimen containers (Thermo Fisher Scientific, Rochester, NY) and processed for enrichment as follows. Ten rehang carcass rinsates and ten post-chill carcass rinsates were set up as controls (uninoculated) to screen for natural contamination. Twenty rehang carcass rinsates and twenty post-chill carcass rinsates were inoculated with 100 µL of bacterial suspension, described above, at 1 to 2 CFU per 30 mL of rinsates. Each of the control and inoculated rinsate was then combined with 30 mL of BPW ISO for the LAMP method and 30 mL of BPW for the PCR method. The samples were gently mixed and incubated at 41.5°C for 18 hours for the LAMP method and at 35°C for 20 hours for the PCR method (Table 1).

### 2.3.3 Raw poultry product preparation

An unpaired study was done to compare the detection of *Salmonella* in raw poultry products (Figure 3) by the LAMP-bioluminescent assay and PCR method. Twelve types of raw products were utilized in this study. Using a pre-heated bead sterilizer and metal cutting utensils, ten 325 g samples were cut from each product type. The products were added to a 4 L sterile Whirl-Pak filter bag (Nasco, Fort Atkinson, WI). Two to three samples out of 5 samples for each product type was used to screen for natural contamination and the rest was inoculated with 100  $\mu$ L of *Salmonella* suspension, described above, at 1 to 2 cfu/325 g test portion, and then stored at 4-8°C for 24 hours before enrichment.

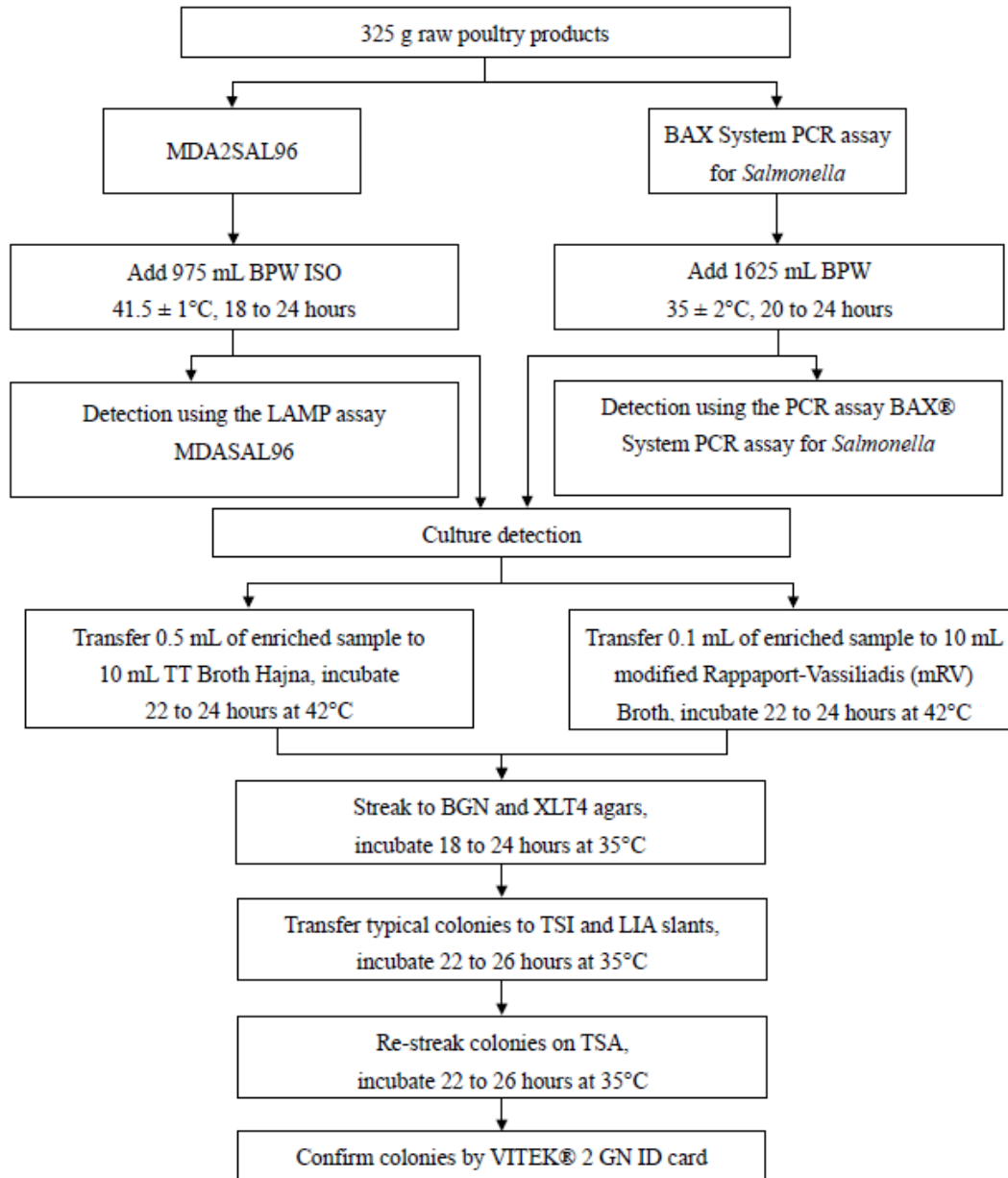


Figure 3. Flow chart for detection of *Salmonella* in raw poultry products by MDA2SAL96 and PCR method and culture confirmation.

### 2.3.4 Raw poultry product testing

For LAMP method, 325 g of sample (uninoculated or artificially contaminated) in a 4 L sterile Whirl-Pak filter bag (Nasco) was combined with 975 mL of BPW ISO, homogenized in a stomacher (Stomacher 3500, Seward Laboratory Systems Inc., Port Saint Lucie, FL) for 30 seconds and incubated at 41.5°C for 14 hours (Table 1).

For PCR method 325 g of sample (uninoculated or artificially contaminated) in a 4 L sterile Whirl-Pak filter bag

(Nasco) was combined with 1,625 mL of BPW, homogenized in a stomacher (Seward Laboratory Systems Inc.) for 30 seconds and incubated at 35°C for 20 hours (Table 1).

## 2.4. *Salmonella* detection

For the LAMP assay, 20 µL of sample after enrichment in BPW ISO (poultry rinsates and parts) or TT broth (boot swabs) was collected and processed for detection using the 3M™ Molecular Detection System following manufacturer's instructions (3M Food Safety). For the PCR method, samples (poultry rinsates and parts) were analyzed after enrichment in BPW by the BAX® System PCR assay for *Salmonella* assay following manufacturer's instructions.

Regardless of the presumptive results obtained by the two methods, primary enrichments from both the LAMP and the PCR method were culture-confirmed per the USDA FSIS Microbiology Laboratory Guidebook (MLG) 4.11 Culture reference method [35] with some modifications (Figures 2, 3). For boot swabs, TT broth enrichments were confirmed following NPIP method (Figure 1).

## 2.5. Analysis of results

The alternative method results (presumptive results) were compared to the culture method to determine false positive and false negative rates [36]. Presumptive results obtained for both carcass rinsates and raw poultry products for *Salmonella* detection with the LAMP and the PCR method were compared with the confirmed culture results and sensitivity (false negative rate) and specificity (false positive rate) [36] was calculated using equation 1 and 2, respectively.

$$\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of true positives} + \text{Number of false negatives}} \times 100 \quad (1)$$

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives} + \text{Number of false positives}} \times 100 \quad (2)$$

True positives: culture-confirmed positive results

True negatives: culture-confirmed negative results

False negatives: presumptive negative results, positive culture results

False positives: presumptive positive results, negative culture results

For rinsate and product samples, Probability of Detection (POD) was computed for both the LAMP method (POD LAMP, POD<sub>2</sub>) and the PCR method (POD PCR, POD<sub>1</sub>) and used as a statistical model to validate LAMP method [37]. POD was calculated as the ratio of number of positives to total number of samples tested for each method at each analyte concentration. dPOD, the differential between the probability of detection (POD) for the LAMP method (POD<sub>2</sub>) and the POD for the PCR method (POD<sub>1</sub>) was computed. The lower and upper confidence limit (95% confidence interval) for POD<sub>1</sub> and POD<sub>2</sub> was calculated and used to calculate the lower and upper limit for dPOD [37]. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level [37].

## 3. Results

### 3.1. Primary production samples

The boot swabs were not artificially inoculated as natural contamination was prevalent in the samples. Detection of *Salmonella* in naturally contaminated boot swabs showed different prevalence of *Salmonella* in the two sampled farms: 8 and 25 positives out of 30 samples were determined from farms A and B, respectively by the LAMP method. Presumptive results from the LAMP method were all culture confirmed and in total agreement with the NPIP method (Table 2).

**Table 2. Detection of *Salmonella* in primary production boot swabs**

Boot swabs <sup>a</sup>	Number of boot swabs	Number of positive samples	
		Presumptive result <sup>b</sup>	Culture Confirmed result <sup>c</sup>
		MDA2SAL96	
Natural contamination ( <i>Salmonella</i> )	30	8	8
Natural contamination ( <i>Salmonella</i> )	30	25	25

<sup>a</sup>Average background aerobic flora varied from 7.7 to 9.38 Log cfu/mL.

<sup>b</sup>Number of positive samples detected by the LAMP method, MDA2SAL96.

<sup>c</sup>All samples were culturally confirmed using NPIP method for *Salmonella* (Figure 1).

## 3.2. Carcass rinsates

### 3.2.1 *Salmonella* detection in Rehang carcass rinsates

The average background aerobic flora in rehang rinsate was about 4.4 Log cfu/mL. Natural contamination of *Salmonella* was detected in 1 out of 10 rehang rinsates by the LAMP method and in 2 out of 10 rehang rinsates by the PCR method. For the 20 inoculated rinsate samples, *Salmonella* was detected in 18 samples analyzed with the LAMP method and in 17 samples for the PCR method (Table 3). Two confirmed samples could not be detected by the LAMP method and three samples by the PCR method. In addition, PCR method had one unconfirmed presumptive result. The sensitivity of the LAMP assay, was 91.3% and 87.5% for the PCR method. Specificity was 100% for the LAMP method and 90% for the PCR method.

In the initial culture screening from the rehang samples, four presumptive positive samples from the LAMP method and three presumptive positive samples from the PCR method were considered to be potential “false positives” as atypical characteristic colonies were seen on selective *Salmonella* agar. The presumptive positives were restreaked on several BGS and XLT4 agar and up to three additional typical and atypical colonies were collected from *Salmonella* selective agar for biochemical identification. With additional colony identification, presumptive positive samples were confirmed as *Salmonella*. Some of the atypical colonies were identified as *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Citrobacter freundii*.

### 3.2.2 *Salmonella* detection in Post-chill carcass rinsates

The average background aerobic flora in post-chill rinsates was about 2.28 Log cfu/mL. No natural *Salmonella* contamination was detected in any of the control (uninoculated) samples, while *Salmonella* was detected in 19 out of the 20 rinsates analyzed for both methods. Presumptive results obtained by both the methods were in 100% agreement with culture confirmation results (Table 3). The sensitivity and specificity of both the methods for the post-chill rinsates was 100%.

**Table 3. Comparison between the LAMP assay and the PCR method for the detection of *Salmonella* in carcass rinsates**

Rinsate <sup>a</sup>	Number of rinsates	Presumptive result <sup>b</sup>	Culture Confirmed result <sup>c</sup>	Number of rinsates	Presumptive result <sup>b</sup>	Culture Confirmed result <sup>c</sup>
Rehang carcass		MDA2SAL96		BAX® System PCR assay for <i>Salmonella</i>		
Natural contamination	10	1	1	10	2	1
<i>Salmonella</i> <sup>d</sup>	20	18	20	20	17	20
Total	30	19	21	30	19	21
Post-chill carcass						
Natural contamination	10	0	0	10	0	0
<i>Salmonella</i> <sup>d</sup>	20	19	19	20	19	19
Total	30	19	19	30	19	19

<sup>a</sup>Average background aerobic flora varied from 3.14 to 4.4 Log cfu/mL for rehang rinsate and 2.5 to 2.28 Log cfu/mL for post-chill rinsate.

<sup>b</sup>Number of positive samples detected by molecular method (MDA2SAL96 for LAMP and BAX® System PCR assay for *Salmonella* for PCR).

<sup>c</sup>All samples were culturally confirmed as outlined in Figure 2.

<sup>d</sup>Rinsates were inoculated with about 1 to 2 cfu/rinsate.

### 3.2.3 Raw poultry products

The average background aerobic flora in various products ranged from 1.3 to 5.9 Log cfu/g (Table 4). One confirmed sample was not detected by the LAMP method (seasoned chicken breast) and two confirmed samples were not detected by the PCR method (chicken thighs and ground chicken). In addition, both methods had one unconfirmed presumptive positive result. Sensitivity for the detection of *Salmonella* in raw chicken products was 96.6% for the LAMP method and 93.3% for the PCR method. The specificity was 97% for both methods.

The 3M™ Molecular Detection System uses an external amplification control, MDMC, to assess sample interference during the DNA isothermal amplification reaction. All matrices evaluated in this study for *Salmonella* detection resulted in a valid result with the MDMC indicating no inhibition of amplification reaction with any of the matrices tested in this study. Similarly, all matrices gave valid internal amplification result with the PCR method (BAX® System PCR assay

for *Salmonella*).

**Table 4. Comparison between the LAMP assay and the PCR method for the detection of *Salmonella* in raw poultry products**

Product <sup>a</sup>	Background flora (Log cfu/g)	MDA2SAL96			BAX® System PCR assay for <i>Salmonella</i>		
		Number of samples	Presumptive result <sup>b</sup>	Confirmed result <sup>c</sup>	Number of samples	Presumptive result <sup>b</sup>	Confirmed result <sup>c</sup>
Ground Turkey	1.90	5	0	0	5	1	1
Marinated Tenders	3.41	5	2	1	5	1	0
Partially fried Chicken Patties	6.36	5	2	2	5	1	1
Partially fried Chicken Nuggets	4.10	5	5	5	5	5	5
Whole Bird without giblets	3.05	5	3	3	5	3	3
Mechanically Separated Chicken	5.43	5	5	5	5	5	5
Seasoned Chicken Breast	4.12	5	2	3	5	3	3
Plain Chicken Breast	3.41	5	3	3	5	0	0
Chicken Tenders	2.08	5	2	2	5	2	2
Chicken Thighs	6.49	5	1	1	5	3	4
Ground Chicken	3.65	5	1	1	5	2	3
Chicken Legs	6.51	5	2	2	5	1	1
Total		60	28	28	60	27	28

<sup>a</sup>Naturally contaminated or artificially inoculated samples with about 1 to 2 cfu/sample of *Salmonella*.

<sup>b</sup>Number of positive samples detected by molecular method (MDA2SAL96 for LAMP and BAX® System PCR assay for *Salmonella* for PCR).

<sup>c</sup>Number of positive samples detected through culture (Figure 3). All samples were culturally confirmed regardless of presumptive results.

### 3.2.4 Data analysis

Analysis of dPOD for carcass rinses and raw poultry products showed that the detection of *Salmonella* spp. with the alternative LAMP method was not significantly different (95% confidence interval) from the PCR method (Table 5).

**Table 5. Probability of detection for unpaired comparison between the LAMP assay and the PCR method for the detection of *Salmonella* in various poultry matrices**

Matrix	Inoculation level <sup>a</sup>	N <sup>b</sup>	Confirmed positives (PCR)	Confirmed positives (LAMP)	POD <sub>1</sub> <sup>c</sup>	POD <sub>2</sub> <sup>d</sup>	dPOD <sup>e</sup>	95% CI <sup>f</sup>	
Carcass rinse (rehang)	Natural	10	2	1	0.2	0.1	0.1	-0.24	0.42
	Low	20	17	18	0.85	0.90	0.05	-0.17	0.27
Carcass rinse (post-chill)	Natural	10	0	0	0	0	0	-0.28	0.28
	Low	20	19	19	0.95	0.95	0	-0.19	0.19
Raw poultry products	Low/Natural	60	27	28	0.45	0.47	0.02	-0.16	0.19

<sup>a</sup>Samples were inoculated with about 1 to 2 cfu/rinsate.

<sup>b</sup>N: Total number of samples analyzed with each of the method.

<sup>c</sup>POD<sub>1</sub>: Probability of Detection for the PCR method.

<sup>d</sup>POD<sub>2</sub>: Probability of Detection for the LAMP method.

<sup>e</sup>dPOD: Differential between the POD<sub>1</sub> and the POD<sub>2</sub>.

<sup>f</sup>95% CI: LCL is the lower confidence level, UCL is the upper confidence level. If the confidence interval (CI) of a dPOD contains zero, then the difference is not statistically significant at the 5% level.

## 4. Discussion

LAMP uses a unique DNA polymerase for continuous DNA amplification that is resistant to matrix interference and inhibitors [15-24]. LAMP assays have the same or higher sensitivity compared to PCR assays and traditional culture methods in detecting foodborne pathogens such as *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, *Campylobacter*



and others from various food matrices [22-24, 26-33, 38, 39].

The LAMP assay (MDA2SAL96) used in this study provided next day results for detection of *Salmonella* in boot swab samples and was comparable (100% agreement) to NPIP culture-based method. Hence, *Salmonella* can be detected by the LAMP method in boot swabs by direct enrichment in TT broth. Similarly, the LAMP assay was able to detect *Salmonella* in rehang and post-chill rinsates and raw poultry products and had higher or equivalent sensitivity and specificity to the PCR method.

In the initial culture screening of rinse and raw poultry products samples, some samples in both methods had atypical colonies on differential selective *Salmonella* agar. Further testing identified some atypical colonies as true *Salmonella*. This illustrates the challenge of isolating *Salmonella* from samples with competitive microflora as the associated microflora are also recovered in selective agars in addition to atypical phenotypes often exhibited by *Salmonella* [40]. Molecular methods based on amplification of specific DNA targets in pathogenic microorganisms are more specific than traditional method that is based on the use of selective agents or biochemical reactions. While colony confirmation is still relevant to laboratory testing, it is also important to recognize the higher specificity of molecular detection methods for pathogen testing [10-14].

*Salmonella* LAMP-bioluminescent assay has been successfully used for *Salmonella* detection in a variety of food samples [26-30, 32, 33]. The results from this study further validate that the LAMP-bioluminescent method is capable of detecting *Salmonella* in poultry related matrices with higher or equivalent sensitivity and specificity to the PCR method used in the study.

New performance standards adopted by USDA FSIS and other government agencies require specific and sensitive methods for detection of *Salmonella* in variety of poultry matrices. The LAMP method used in this study enables rapid detection of *Salmonella* in matrices commonly analyzed by the poultry industry. Recently, the USDA FSIS has updated Microbiology Laboratory Guidebook (MLG) to include 3M™ Molecular Detection Assays for *Salmonella* [35] and *Listeria monocytogenes* [41] giving further validation to LAMP assays as suitable alternative to PCR assays.

## 5. Conclusions

This study evaluated the performance of a LAMP-bioluminescent assay for the detection of *Salmonella* in primary production samples, poultry rinses and raw poultry products compared to a PCR method. Samples were tested after enrichment by LAMP-bioluminescent assay and PCR method. The alternative LAMP-bioluminescent assay was able to detect *Salmonella* in the various matrices tested and had higher or equivalent sensitivity and specificity to the PCR method used. There was no significant difference (95% confidence interval) between the LAMP and PCR method for detection of *Salmonella* in poultry matrices tested. Hence, the *Salmonella* LAMP-bioluminescent assay is an acceptable alternative to the PCR method for rapid detection of *Salmonella* in variety of poultry matrices.

## 6. Acknowledgements

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## 7. Conflict of interest

The authors, R. Rajagopal, G. Lopez-Velasco and J. M. David are employees of 3M Food Safety which offers multiple commercial solutions, including 3M™ Molecular Detection System and 3M™ Molecular Detection Assays to the food industry. Melissa Sisemore and Jamie Goseland are employees of WBA Analytical Laboratory. WBA Analytical Laboratories provides microbiological and chemical analysis, product research and development, and technical services to its customers.

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