Determination of the optimal pre-enrichment period for the detection of *Salmonella enterica* in artificially inoculated meat products using culture, PCR and LAMP assays

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Determination of the minimum enrichment period and most suitable detection assay for *Salmonella* in meat is essential in food safety and prevention of *S. enterica*-related outbreaks. Using artificially inoculated raw and processed meat products, the optimal pre-enrichment period for culture, polymerase chain reaction (PCR), and loop-mediated isothermal amplification (LAMP) assays to detect *Salmonella* in meat was evaluated. Optimal pre-enrichment period is herein defined as the shortest period of incubation to reliably detect the target organism. The three detection assays were conducted in a time-point manner, specifically the first six hours and 24th hour of pre-enrichment and the 48th hour of selective enrichment phase. LAMP assay had an optimal pre-enrichment period of 24 hours, i.e. 100% detection, on beef, chicken, pork, and bacon. Alternatively, the PCR assay had an optimal enrichment period of 48 hours covering the complete pre-enrichment and selective enrichment phases. The culture assay failed to achieve 100% detection, even after a 24-hour incubation or end of pre-enrichment period but had a higher tolerance to inhibitory substances than PCR. Of the three detection assays evaluated, LAMP assay had the shortest optimal enrichment period, highest sensitivity to *Salmonella* in meat products, and highest tolerance to inhibitory substances.

KEYWORDS

culture assay, food safety, loop-mediated isothermal amplification (LAMP) assay, meat, polymerase chain reaction (PCR), enrichment period, *Salmonella enterica*

INTRODUCTION

Salmonella is one of the most important pathogens affecting both humans and animals. It causes food-borne diseases and outbreaks that result in major economic losses in the food industry. The World Health Organization (2018) has estimated that typhoidal *Salmonella* infects 11–20 million people, with 128,000 to 161,000 deaths every year, and has declared non-typhoidal *Salmonella* as one of the four key causes of diarrheal diseases worldwide. *Salmonella* infection in humans may lead to gastroenteritis, systemic infection, and death. On the other hand, infected animals serve as reservoir for transmission of the pathogen to humans. Infection in animals, moreover, can lead to major economic losses due to lower productivity brought about by animal morbidity and higher cost of disease treatment (Nowak et al. 2007).

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In the Philippines, data on the incidence of *Salmonella* can be found mainly on reports of food poisoning outbreaks and animal infections. *Salmonella* has been identified as one of the leading causes of food-borne infections from 1995–2004 during which majority of *Salmonella*-related outbreaks originated from contaminated meat-containing dishes with spaghetti as the leading food vehicle (Azanza 2006). Only a few studies have reported *Salmonella* in animals or food as unrelated to any outbreak or infection. Other studies have detected *Salmonella* in meat and egg samples from markets, abattoirs, and farms by using the conventional culture technique (Balala et al. 2006; Vizmanos and Baldrias 1998) and polymerase chain reaction (PCR) (Calayag et al. 2017; Ng and Rivera 2015; Soguilon-Del Rosario and Rivera 2015). The latest local study, which utilized the latter technique, estimated that 57.64% of 720 samples of retail meat were contaminated with *Salmonella* (Santos 2017).

The culture technique, standardized by the International Organization for Standardization (ISO) 6579:2002 and the United States Food and Drug Administration (USFDA), is the conventional method for the identification and detection of *Salmonella*. It includes pre-enrichment, selective enrichment, selective plating, and biochemical and serological tests. Although it is a reliable technique, it is complicated, labor-intensive, expensive, and time-consuming as it takes five to seven days to complete the process (Zhang et al. 2011). These disadvantages have prompted the development of rapid screening and detection methods for *Salmonella*. Molecular methods, such as the end-point PCR and quantitative real-time PCR, which are specific and sensitive assays and which have been evaluated, have emerged as complementary or alternative methods. In March 2018, a real-time quantitative PCR assay protocol was added in the Bacteriological Analytical Manual (BAM) as an alternative confirmatory test of foodborne *Salmonella* isolates in leafy produce, herbs, and sprouts (Andrews et al. 1998). The use of PCR-based methods, however, are limited by factors like requirement of expensive equipment and reagents, sensitivity to inhibitory and/or contaminating chemicals, and complicated downstream analysis (Kokkinos et al. 2014). Another molecular technique with great potential for use in rapid, sensitive, and specific detection is the loop-mediated isothermal amplification (LAMP) assay developed by Notomi et al. (2000). This technique works under isothermal conditions between 60–65 °C, consequently eliminating the need for thermocycler, and is resistant to changes in physiochemical parameters like temperature, pH, and biological substances (Kaneko et al. 2007). Complex primer design, high sensitivity to contaminants, and the absence of internal amplification controls are some of the factors that limit the application of LAMP (Deguo et al. 2008). Despite these limiting factors, LAMP-based assays have been used in many studies in the detection of contaminants, including *Salmonella* in different food matrices (Kokkinos et al. 2014), and have emerged as practical alternatives to the conventional culture and PCR-based methods. These studies typically involve a tandem of LAMP assay and pre-enrichment and/or selective enrichment of culture but with shorter incubation period (Li et al. 2015; Srisawat and Panbangred 2015; Techathuvanan et al. 2010; Ye et al. 2011; Zhang et al. 2012). In summary, culture assay is the conventional method, PCR assay is a widely-practiced molecular detection method, while LAMP assay is a promising thermocycler-independent and rapid molecular method for the detection of *Salmonella*.

Determination of the optimal minimum incubation period for *Salmonella* detection may contribute to shorter than normal turnaround time without compromising the accuracy of the detection assay, the end result of which would be fresh and safe meat products. The optimal pre-enrichment periods for *S. enterica* in meat products to become detectable in the culture, PCR, and LAMP assays were determined in this study. Raw (i.e. pork, chicken and beef) and processed (i.e. bacon and sausage) meat products were artificially inoculated with representative *Salmonella* isolates belonging to different serovars. Detection of *Salmonella* was done hourly during the first six hours of pre-enrichment and at the 24th hour of pre-enrichment and 48th hour of selective enrichment periods.

MATERIALS AND METHODS

Salmonella Strains

Salmonella strains with putative serovar identities *S.* Typhimurium, *S.* Enteritidis, and *S.* Anatum (see Table S1) were isolated from retail meat collected from wet markets and characterized using a modified molecular serotyping protocol (Agron et al. 2001; Cardona-Castro et al. 2009; Echeita et al. 2002; Herrera-León et al. 2004; Hirose et al. 2002; Hong et al. 2008; Widjojoatmodjo et al. 1991). The strains were cultured in tryptic soy broth at 35 °C for 24 h, harvested by centrifugation at 10,000 × g for 2 min then adjusted to a cell density of about 1.5×10^8 cells in phosphate-buffered saline (PBS) using 0.5 McFarland standard.

Artificial Inoculation in Meat

Raw meat products namely pork, chicken, and beef; and processed meat products bacon and sausage were purchased and immediately transported to the laboratory for processing. Surface decontamination of meat was performed in the following sequence: dipping in 3% lactic acid solution at 75 °C for 20 s, followed by immersion in 80 °C sterile hot water for 20 s (Pichpol 2009), and lastly, drying and exposure to ultraviolet (UV) light for 15 min. After decontamination, 25 g of meat was cut into finer pieces and placed into sterile containers with 225 mL sterile buffered peptone water (BPW) spiked with *Salmonella* to reach a final concentration of ~0.2 cells/mL. The three *Salmonella* serovars were used singly in culture spiking. Surface-decontaminated meat incubated in sterile BPW served as the uninoculated or negative control. Artificial inoculation was done in triplicates for each meat type.

Culture Assay

The succeeding culture detection and enumeration of *Salmonella* is a modified version of the protocol in BAM (Andrews and Hammack 2007). Pre-enrichment was conducted by incubating the meat-BPW mixture at 35 °C for 24 h. For every hour during the first six hours of the pre-enrichment, 1 mL of the mixture was transferred into a tube for DNA extraction and 0.1 mL was plated onto xylose lysine deoxycholate (XLD) agar. At the 24th hour or end of pre-enrichment, 1 mL was collected for DNA extraction, 0.1 mL was diluted then plated onto XLD agar, and 0.1 mL was transferred to 10 mL of Rappaport-Vassiliadis (RV) broth for selective enrichment. The RV broth was incubated at 42 °C for 24 h after which the cells were collected by centrifugation. The XLD agar plates were incubated at 35 °C for 24 h. The presence of typical *Salmonella* colonies in XLD characterized by pink coloration with or without black centers was noted. Samples collected from BPW and RV mixtures for DNA extraction were pre-processed by harvesting cells through centrifugation and washing with PBS. The samples were subsequently stored in freezer until use.

DNA Extraction

Boil lysis method was adapted for the extraction of DNA (Wang et al. 2008). Briefly, collected cells in tubes were washed with PBS by centrifugation at 15,330 × g for 5 min. The supernatant was discarded and 100 µL of Tris-EDTA (TE) buffer was added. The cells were then boiled for 10 min followed by centrifugation at 6,000 rpm for 5 min. The supernatant containing the DNA was transferred into sterile tube and stored in -20 °C until use.

Table 1: *Salmonella* detection in pork, chicken, beef, bacon and sausage at variable enrichment periods using three different methods: culture (C), PCR (P) and LAMP (L). The data in each period for the uninoculated meat products were derived from three independent trials (n=3). Alternatively, the data in each period for the *Salmonella*-inoculated meat products (n=9) were generated from three independent trials of each of the three *Salmonella* serovars used. Notation: -, +, ++, and +++ indicate 0%, 1-49%, 50-99%, and 100% relative frequency of positive detection, respectively, while nt indicates not tested.

Treatment	Enrichment	Pork			Chicken			Beef			Bacon			Sausage		
	time (hour)	C	P	L	C	P	L	C	P	L	C	P	L	C	P	L
Decontaminated,	0	+	-	+	+	-	+	+	-	+	+	-	++	+	-	++
Salmonella-	1	+	-	++	+	-	+	+	-	++	+	-	++	+	-	+++
inoculated	2	+	-	++	+	-	+	+	-	++	+	-	++	+	-	++
	3	+	-	++	+	-	+	+	-	++	+	-	++	+	-	+
	4	+	-	++	+	-	+	+	-	++	+	-	+	+	-	++
	5	++	-	++	+	-	++	+	-	+	+	-	++	+	+	++
	6	++	+	++	++	+	++	++	+	+	+	+	++	++	+	+++
	24	+	++	+++	++	++	+++	++	++	+++	++	++	+++	++	++	++
	48	Nt	+++	+++	nt	+++	+++	Nt	+++	+++	nt	+++	++	Nt	+++	+++
Decontaminated,	0	-	-	+	-	-	++	-	-	++	-	-	+++	-	-	+++
Uninoculated	24	-	-	++	+	-	+++	+	-	+++	+	-	+++	+	-	+++

Epoch™ Microplate Spectrophotometer was used to determine the quantity (A_{260}) and quality ($A_{260/280}$) of the DNA extracts. For each *Salmonella* serovar in each meat, the extract with highest quality ($A_{260/280} \approx 1.8$) and quantity was used as template in the succeeding DNA amplification techniques.

PCR Assay

The primers targeting the *invA* gene of *S. enterica* designed by Chiu and Ou (1996) was utilized in the PCR assay. The PCR reaction mixture (12.5 µL) consisted of 1× GoTaq® PCR master mix, 0.4 µM of each primer, nuclease-free water, and 1 µL DNA template. The PCR condition was as follows: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 2 min, and final extension at 72 °C for 5 min. Resolution of the amplicons was performed by electrophoresis in 1.5% agarose gel with 10,000× SYBR® Safe DNA Gel Stain followed by visualization using UV trans-illuminator. Samples presenting ~300bp amplicon were considered positive for *Salmonella*. Three independent trials were performed for each meat type and with each *Salmonella* serovar.

LAMP Assay

The LAMP primers targeting the *invA* gene of *S. enterica* designed by Hara-Kudo et al. (2005) was utilized in this assay. The LAMP reaction mixture (25 µL) consisted of 1× Thermopol® reaction buffer, 6 mM $MgSO_4$, 1.4 mM each of dNTPs, 1.6 µM each of inner primers, 0.2 µM each of outer primers, 0.4 µM each of loop primers, nuclease free water, and 1 µL DNA template. Initial denaturation was performed at 95 °C for 2 min prior to addition of 320 U/mL *Bst* DNA Polymerase, Large Fragment to each tube. LAMP was performed by incubation at 65 °C for 1 h then terminated by heating to 80 °C for 2 min. Assessment of LAMP in-tube and in-gel was performed. Each tube was added with 1,000× SYBR® Safe DNA Gel Stain for the in-tube assessment while agarose gel electrophoresis was performed as described above. Samples that fluoresce (in-tube) or show smear (in-gel) under UV light were considered positive for *Salmonella.* Three independent trials were performed for each meat type and with each *Salmonella* serovar.

Sensitivity of Molecular Detection Assays

The adjusted cell density of *S.* Enteritidis strain was serially-diluted ten-fold in PBS to determine the sensitivity of the PCR and LAMP molecular detection assays. The sensitivity test covered 10^8 to 10^0 cells/mL. Each assay was performed in triplicates. Only the putative *S.* Enteritidis strain was used as representative of *Salmonella* spp. in these assays.

RESULTS

The detection of *Salmonella* in inoculated and uninoculated decontaminated meats underscored the effectiveness of the decontamination method and the sensitivity of the detection assays. The effectiveness of the decontamination protocol was dependent on the detection method (Table 1). Factors, like sensitivity, specificity and robustness of the detection method determined the level of effectiveness of a decontamination protocol. For instance, an assay with low specificity and low sensitivity is likely to indicate that a decontamination protocol is effective even if it is not. No *Salmonella* was detected by both culture and PCR assays in the uninoculated meat at 0 h. At the end of pre-enrichment incubation of the uninoculated meat, no *Salmonella* was detected by PCR. However, typical *Salmonella* colonies were observed in XLD agar. LAMP assays in uninoculated meat detected *Salmonella* at 0 h and 24 h incubation on all meat types. Notably, all trials in all set-ups tested positive in LAMP assay at 0 h incubation of uninoculated processed—Filipino-style bacon and sausage meat samples.

In the time-point detection of *Salmonella* (Table 1) in inoculated meat where at least 0.2 cells/mL was present at 0 h incubation,

the LAMP assay had the highest frequency of positive detection on almost all time-points. The relative frequencies of positive detection specific for each *Salmonella* serovar in each meat type are presented in Table S2. Of the incubation periods tested, 100% detection of *Salmonella* in raw meat samples and bacon was achieved at the 24 h pre-enriched sample using LAMP. With PCR assay, 100% detection was achieved at the 48-h incubation or at the end of the selective enrichment. PCR was not able to detect *Salmonella* in raw meat and bacon until the 6th hour of pre-enrichment. With culture assay done by plating in XLD until the 24th hour of pre-enrichment, typical *Salmonella* colonies were observed starting at 0 h incubation. However, 100% detection by culture assay was not achieved on all meat types.

In comparison with the aforementioned performance of LAMP and PCR in BPW and RV culture media, the sensitivity assays for LAMP and PCR using cells in PBS indicated that the minimum detectable cell densities were 10^0 cell/mL and 10^6 cells/mL, respectively.

DISCUSSION

Meat Decontamination

Salmonella infection is a rising global health concern due to the high and increasing number of non-typhoidal *Salmonella* in humans. About 10^5 *Salmonella* cells can cause infection in a human host although highly susceptible individuals can have salmonellosis with as low as 15–20 cells. Most infections in humans have been attributed to consumption of contaminated food products (Kokkinos et al. 2014). On the other hand, *Salmonella* is a major concern in the animal industry due to reduced productivity (Nowak et al. 2007). Hence, the use of rapid and sensitive detection method for *S. enterica* is essential for food safety and prevention of *S. enterica*-related outbreaks.

This study evaluated the performance of three detection assays at different pre-enrichment periods of *Salmonella* in raw and processed meat products. Removal or reduction of natural bacterial flora in meat was performed prior to inoculation with *Salmonella*. The adapted meat decontamination protocol by Pichpol (2009) was chosen based on zero to minimal effect on the quality of meat for food preparation. The quality of meat is of concern since color, odor, and taste might negatively be affected by physical and chemical decontaminants depending on the concentration, temperature, and treatment of choice. Together with the ability to reduce microbial load, adverse effects of the decontamination procedure to the quality of meat should be negligible (James et al. 2000).

Treatment using 3% lactic acid at 75 °C had no adverse effect on the surface and odor of the meat, a finding which is in agreement with the observations of Pichpol (2009). Lactic acid at 1–3% concentration was considered as optimal when taking into consideration the quality and safety of food (Kanellos and Burriel 2005). Lactic acid and other organic acids are generally recognized as safe (GRAS) by the USFDA and are applicable for use in meat and meat products (Sohaib et al. 2016). Among the organic acids commonly used as meat decontaminants, lactic acid has better efficacy than acetic acid in the complete inactivation of *Salmonella* Typhimurium DT 104 and reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes* (Samelis et al. 2001) and both Gram-negative and Gram-positive bacteria in meat products (Castillo et al. 2001; Kanellos and Burriel 2005). Additionally, acetic acid has been observed to be an effective meat decontaminant although it confers strong flavor to the meat (Skandamis et al. 2010).

Slightly lighter color of meat surfaces was evident after treatment with lactic acid and hot water similar to the observation of Pipek et al. (2005). When combined with hot water, the effect of lactic acid is heightened (Pipek et al. 2004b; Siragusa 1995). Earlier studies have proven the greater reduction effect of hot water and lactic acid using beef (Kang et al. 2001; Ozdemir et al. 2006) and pork (Castelo et al. 2001) with unnoticeable negative effects on the quality of meat. Synergism between acids and heat has been linked to the heightened effect of the combination treatment as cell injuries might have resulted upon treatment with physical methods such as hot water, thus, increasing sensitivity to acids (Koutsoumanis et al. 2004). The observed slight reduction of skin redness was suggested as caused by the decrease of pH and removal of heme pigments from blood residues that were washed off from the surface (Pipek et al. 2005). Nonetheless, the color change observed was considered insignificant. Surface changes that are possibly induced by lactic acid, such as protein denaturation and change of functional groups dissociation, are beneficial in preventing weight loss since protein denaturation causes closing of pores subsequently reducing water evaporation from the meat surface (Pipek et al. 2004a). Furthermore, flavor and color of food can be enhanced (Sohaib et al. 2016) while increase of spoilage microorganisms can be hindered and production of adverse chemicals can be prevented with lactic acid treatment (Smaoui et al. 2012).

Notably, the effectiveness of the decontamination protocol is dependent on the detection assay used. It has been known through numerous studies that culture assay is specific, PCR is specific and sensitive, and LAMP is specific, sensitive, and robust. Based on the results on unspiked meat, LAMP is the most sensitive detection assay, followed by culture and PCR techniques. The decontamination protocol was effective as shown by the PCR and culture assay results while LAMP results indicated the contrary.

Salmonella Detection in Decontaminated, *Salmonella*-spiked Meat

At the pre-enrichment period, the decontaminated meat samples were inoculated with *Salmonella* to a final concentration of about 0.2 cells/mL, the minimum cell density of *Salmonella* per tube tested for LAMP sensitivity by Hara-Kudo et al. (2005) and which was considered as low inoculation level by Bird et al. (2013). Previously-isolated *Salmonella* serovars commonly reported in clinical isolates (*S.* Enteritidis and *S.* Typhimurium) and retail meat products (*S.* Anatum) in the Philippines were used as representative foodborne *Salmonella* for the artificial contamination. *Salmonella* Typhimurium and *S.* Enteritidis usually cause mild enteric diseases on wide range of host animals, such as humans, livestock, domestic chickens, rodents, and birds (Uzzau et al. 2000). These serovars can cause localized self-limiting enterocolitis in immunocompetent humans and systemic infections in immunocompromised individuals (Coburn et al. 2005). *S.* Anatum is widely distributed among different host animals (Almaden and Wahlin 1946) and commonly isolated in retail meat products in the United States (Doyle et al. 2013) and the Philippines (Santos 2017). The infection it causes in humans is frequently associated with gastroenteritis and with septicemia, chlolecystitis, and meningitis although these are rarely reported (Almaden and Wahlin 1946).

The highest relative frequency of positive detection of *Salmonella* in inoculated meat was observed with LAMP assay followed by culture and PCR techniques (Table 1). The performance of the three assays was similar for both *Salmonella*-spiked and uninoculated meats. The optimal incubation for PCR-based detection of *Salmonella* in meat was 48 hours, which included the pre-enrichment and selective enrichment phases of

the culture assay. The PCR assay needed to reach the selective enrichment phase to attain 100% detection. This suggests that a PCR-based detection of *Salmonella* in variable meat types requiring at least about 51 hours for completion is reliable and quick since plating in selective agars and confirmatory tests are eliminated. However, the high number of cells in PBS and the zero to low relative frequency of positive detection in the first hours of incubation of *Salmonella*-spiked meat indicate and support the requirement for high amount of template and the sensitivity to inhibitory substances in the PCR assay (Hyeon et al. 2010; Kokkinos et al. 2014).

In the culture assay, typical *Salmonella* colonies were observed starting at 0 h incubation of inoculated samples suggesting higher tolerance to inhibitory substances of plating in XLD than with PCR. The culture assay failed to achieve 100% detection on all time points, where only typical colonies were considered positive since the strains usually develop only as typical colonies during the revival phase. This indicates its lower sensitivity than the PCR and LAMP assays. Moreover, the nonselective nature of the pre-enrichment medium, which was plated directly on XLD, may have contributed to the observed low detection in the culture assay. In addition to pre-enrichment time, this assay required 18-24 hours for incubation of XLD plates. The hours needed to generate the results were much longer than those of the PCR and LAMP assays.

The LAMP assay, with the highest relative frequency of positive detection on almost all time-points, is the most sensitive and robust detection method since it was able to detect *Salmonella* at the early hour of incubation in culture medium (Table 1) and at very low cell density in PBS. This is in agreement with studies indicating high sensitivity of LAMP as well as its robustness to potential inhibitors that may be present in the culture medium (Kaneko et al. 2007). It reached 100% detection of *Salmonella* in raw meat and bacon at the end of the pre-enrichment period. The optimal incubation period for a reliable detection of *Salmonella* in raw meat using a tandem of culture and LAMP assays is 24 h. Adaptation of this protocol could save resources since only the pre-enrichment phase of culture assay is required. Further, this protocol could save time with an estimate of at least 27 hours for completion, i.e. by adding 3 hours to the pre-enrichment period for the execution of DNA extraction and LAMP. Additionally, great precaution must be exercised when conducting LAMP since it is highly prone to contamination that may lead to inaccurate results as reported by Tomita et al. (2008). Nonetheless, LAMP has higher tolerance against inhibitors compared to PCR and culture assays (Techathuvanan et al. 2010; Yang et al. 2013). It must be noted that only DNA extracts of high quality and quantity were used in the molecular detection assays due to observed inconsistencies in previous experiments using LAMP (unpublished data). Inconsistencies may also be due to the various compositions of the tested food matrices especially processed meat products. Filipino-style bacon is composed of lean pork, table salt, white/brown sugar, sodium phosphate, anisette liqueur, pineapple juice/rice vinegar, garlic, pepper, sodium ascorbate, red food coloring, and potassium nitrate. On the other hand, Filipino-style sausage consists of ground fatty pork, garlic, ground peppercorn, sugar, soy sauce, vinegar, bay leaf, anise seeds/anisado wine, hog casing, table salt, potassium nitrite, and sodium phosphate. These ingredients and the preparation of the tested processed meat samples may play a role in the inconsistencies of *Salmonella* detection across variable incubation hours as well as in the observed 100% *Salmonella* detection in the uninoculated processed meat products. The presence of salt in the processed meat samples may have affected the attachment of *Salmonella,* which is essential to its survival, in terms of prevention of cell injury and cell death, increased resistance to various disinfectants and heat tolerance. The *Salmonella*-collagen fiber interaction is mediated by the physiological relationship between collagen and its ionic environment. The alteration of ionic environment allows the removal of majority of microorganisms from the collagen fibers, which is the preferred attachment site of *S.* Typhimurium (Benedict et al. 1991). Moreover, the fimbriae and motility status of *Salmonella* were not involved in its attachment to animal tissues (Thomas and McMeekin 1981).

Of the three detection techniques evaluated, LAMP assay has the highest sensitivity to *Salmonella* and tolerance to inhibitory substances. On the other hand, PCR assay has higher sensitivity to *Salmonella* but lower tolerance to inhibitory substances than the culture assay. The optimal pre-enrichment period for LAMP-based detection of *Salmonella* in meat is 24 h while PCR-based assay requires both pre-enrichment and selective enrichment of *Salmonella.* A tandem of culture with LAMP and PCR for reliable detection of *Salmonella* in meat would take about 27 h and about 51 h, respectively. However, determination of the detection performance of LAMP and PCR in the 7th to 23rd h of pre-enrichment may lead to reduction of the estimated turnaround time for *Salmonella* detection in meat.

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CONFLICTS OF INTEREST

CAC Justo, MRF Mapile, PDM Santos, and WL Rivera declare that they have no conflicts of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

PDM Santos and WL Rivera conceived the study; CAC Justo and PDM Santos designed the study protocol; CAC Justo and MRF Mapile performed the experiments; WL Rivera received the funding for the study. All authors contributed to data analysis and manuscript writing.

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APPENDIX

Table S1: Molecular serotyping of *Salmonella* isolates used in this study.

Isolate name	Detected genes using Molecular typing				Antigenic formula	Putative serotype identity
	O-serogrouping	H-typing I	H-typing II	Others		
GB06R1B	O:3	*fliC-e,h*	*fljB-1,6*	-	3:eh:1,6	Anatum
QC13R2B	O:9	*fliC-G*	-	*sdf-I*	9:g:- (*Sdf-I*:+)	Enteritidis
BBu03R1B	O:4	*fliC-i*	*fljB-1,2*	-	4:i:1,2	Typhimurium

Table S2: Relative frequency of positive detection at variable time points of three detection methods: culture (C), PCR (P), LAMP (L) for *S.* Anatum, *S.* Enteritidis, and *S.* Typhimurium in pork, chicken, beef, bacon and sausage. Notation: -, +, ++, and +++ indicate 0%, 33%, 67%, and 100% relative frequency of positive detection, respectively, while nt indicates not tested.

Treatment	Enrichment	Pork			Chicken			Beef			Bacon			Sausage		
	time (hour)	C	P	L	C	P	L	C	P	L	C	P	L	C	P	L
Decontaminated,	0	+++	-	+	+++	-	+	+++	-	+	+++	-	+++	+++	-	+
S. Anatum-spiked	1	+++	-	+	+++	-	+	+++	-	++	+++	-	++	+++	-	+++
	2	+++	-	++	+++	-	+	+++	-	++	+++	-	+++	+++	-	+
	3	+++	-	+++	+++	-	+	+++	-	++	+++	-	+++	+++	-	+++
	4	+++	-	+++	+++	-	++	+++	-	+++	+++	-	+	+++	-	++
	5	+++	-	+	+++	-	+++	+++	-	+++	+++	-	+++	+++	++	+++
	6	+++	+++	+++	+++	+	+++	+++	++	+++	+++	++	+++	+++	+++	+++
	24	++	+++	+++	++	+++	+++	++	+++	+++	++	+++	+++	+++	+++	+++
	48	nt	+++	+++	Nt	+++	+++	nt	+++	+++	nt	+++	++	nt	+++	+++
Decontaminated, *S.*	0	-	-	-	-	-	-	-	-	+	-	-	++	-	-	++
Enteritidis-spiked	1	-	-	++	-	-	-	-	-	-	-	-	-	-	-	+++
	2	-	-	++	-	-	-	-	-	+	-	-	-	-	-	+++
	3	-	-	++	-	-	-	-	-	+	-	-	++	-	-	-
	4	-	-	++	-	-	+	-	-	-	-	-	-	-	-	-
	5	-	-	+	-	-	+	-	-	-	-	-	-	-	-	++
	6	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+++
	24	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	++	++
	48	nt	+++	+++	Nt	+++	+++	nt	+++	+++	nt	+++	+++	nt	+++	+++
Decontaminated,	0	-	-	+++	-	-	+++	-	-	++	-	-	++	-	-	++
S. Typhimurium-spiked	1	-	-	++	-	-	+++	+	-	+++	-	-	+++	-	-	+++
	2	-	-	++	-	-	+++	-	-	++	-	-	++	-	-	++
	3	-	-	++	-	-	+++	-	-	++	-	-	+++	-	-	+
	4	+	-	+++	-	-	+	-	-	++	-	-	+++	-	-	+++
	5	+++	-	+++	+	-	+++	+	-	-	+	-	+++	-	-	+++
	6	+++	-	+++	+++	-	+++	+++	-	+	+	-	+++	+++	-	+++
	24	-	+	+++	+++	-	+++	++	++	+++	-	++	+++	++	-	+++
	48	nt	+++	+++	Nt	+++	+++	nt	+++	+++	nt	+++	+++	nt	+++	+++
Decontaminated,	0	-	-	+	-	-	++	-	-	++	-	-	+++	-	-	+++
Uninoculated	24	-	-	++	+	-	+++	+	-	+++	+	-	+++	+	-	+++