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Research Article
Loop-Mediated Isothermal Amplification vs. Guobiao Standards Method for Detection of *Salmonella* in Yoghurt and Yoghurt-Based Drinks

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Abstract

Background and Objective: *Salmonella* has emerged as an important pathogen in several products beyond traditional poultry products. *Salmonella* is not considered a risk in fermented foods due to low pH and the presence of probiotic organisms. However, *Salmonella* can survive in these products and grow given appropriate conditions. It is essential to detect rapidly and accurately contamination of *Salmonella* in these products. The objective of this study was to optimize growth conditions for *Salmonella* in yoghurt and yoghurt-based drinks and compare the Loop-mediated Isothermal Amplification (LAMP) assay to the traditional Guobiao standards (GB) 4789.4-2016 method for *Salmonella* detection. **Materials and Methods:** Twenty-five grams of different types of yoghurt were inoculated with various levels of *Salmonella* and enriched in Buffered Peptone Water (BPW) ISO (1:10 and 1:20 dilution) at 41.5°C for 24 hrs. For control, uninoculated samples were enriched similarly. All the samples were analyzed with a *Salmonella* LAMP-bioluminescent assay and culture-confirmed using GB 4789.4-2016 method. **Results:** *Salmonella* failed to grow to detectable levels in yoghurt samples with 1:10 enrichments in BPW ISO even at high levels of artificial contamination (1000 CFU/25 g). The pH was reduced to 4.2-4.3 after enrichment. However, with 1:20 BPW ISO enrichments, *Salmonella* grew to detectable levels at low spike levels (about 3 CFU/25 g) and was detected by both methods. **Conclusion:** The alternative LAMP assay enabled reliable and rapid detection of *Salmonella* in yoghurt and yoghurt-based drinks providing next-day results compared to 3 to 5 days for the GB method.

Key words: *Salmonella*, LAMP, GB method, yoghurt, rapid detection, buffered peptone water, foodborne

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella, a Gram-negative bacterium, is globally recognized as a major cause of foodborne infection in humans. CDC estimates that 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]. Acute Gastrointestinal Illness (AGI) is a significant burden in China with about 748 million cases of AGI and 420 million healthcare visits each year[2]. It was estimated that about 209 million cases of the foodborne disease occurred in China in 2010-2011[2]. The most common foodborne pathogens involved in outbreaks in China are *Salmonella* species, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and diarrheagenic *Escherichia coli*[3], with *Salmonella* being the main target pathogen detected through microbiological food safety surveillance[4].

Though *Salmonella* is primarily associated with poultry, it can contaminate a variety of food such as meat, eggs, milk, seafood, vegetables, fruits and even chocolate, ice cream and peanut butter[1]. However, there are limited studies on *Salmonella* growth and detection in fermented products. It is generally believed that *Salmonella* and other foodborne pathogens do not survive the fermentation process and do not pose a threat to human health. However, *Salmonella* has been shown to survive in low acid foods such as juices and fermented products such as yoghurt[5-10]. Although no outbreak of *Salmonella* has been linked to yoghurt, there is a need for rapid and cost-effective methods to detect *Salmonella* in fermented products such as yoghurt and yoghurt-based drinks to ensure the safety of these products.

China is a growing market for both food production and consumption and the dairy industry has been growing over the years. China yoghurt segment revenue accounted for more than USD 37 million in 2020 and CAGR is expected at 5.1% between 2020-2025[11]. With the enactment of the 2015 Food Safety Law of the People's Republic of China, prepackaged foods including general food and infant food need to comply with the quality and hygienic test requirements in the applicable Chinese National Food Safety (Guobiao, GB) Standards[12]. Yoghurt belongs to the category of fermented milk in China with a minimum level of 1×10^6 (CFU g^{-1}) of lactic acid bacteria and titratable acidity of >70°T[13]. According to GB standard (GB 19302-2010), yoghurt is a product made of raw cow milk or goat milk or dry milk through a procedure of pasteurization and fermentation with *Streptococcus thermophilus* and *Lactobacillus bulgaricus*[13]. Per GB standard (GB 19302-2010) for fermented milk, the yoghurt samples have zero tolerance for *Salmonella* in 25 g samples[13]. The traditional GB 4789.4-2016 culture method for *Salmonella* detection requires 3-5 days[14]. Although fermented products such as yoghurt pose a low risk to consumers, effective control measures are critical to prevent foodborne infections and rapid detection methods enable quicker action to prevent foodborne outbreaks.

Loop-mediated Isothermal Amplification (LAMP) can amplify DNA under isothermal conditions (60-65°C) with high specificity and sensitivity in 60 min or less[15-19]. 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[15-19]. An extension of LAMP, LAMP-bioluminescent assay, utilizes LAMP for DNA amplification and bioluminescence for the detection of amplified products[20]. Both amplification and detection occur simultaneously and continuously during the exponential phase providing real-time results and a short run time. The *Salmonella* LAMP-bioluminescent assay, 3M Molecular Detection Assay 2-*Salmonella* (MDA2SAL) has been used for the detection of *Salmonella* in a variety of food matrices[21-25] 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 yoghurt and yoghurt-based drinks manufactured in China as compared to culture confirmation by the GB 4789.4-2016 method.

MATERIALS AND METHODS

Study area: The study was conducted at 3M China Research and Development Center in Shanghai, China in 2019 and 2020.

Inoculum preparation: *Salmonella enterica* serovar Paratyphi Type B (CMCC 50094, National Center for Medical Culture Collection, Beijing, China) and *E. coli* (ATCC 25922, American Type Culture Collection, Manassas, VA, USA) isolates were used in this study. The strains obtained were streaked onto nutrient agar and incubated for 24 hrs at 37°C. To prepare *Salmonella* or *E. coli* inoculum, an isolated colony from nutrient agar plate was inoculated into 100 mL of brain heart infusion broth (Beijing Land Bridge Technology Co. Ltd., Beijing, China) using a sterile inoculating loop and incubated

for 24 hrs at 37°C. After incubation, serial 10-fold dilutions of cultures were prepared in buffered peptone water (BPW, 3M Food Safety, St. Paul, MN) and plated on 3M Petrifilm Aerobic Count Plate (3M Food Safety) and incubated at 37°C for 24 hrs. The colonies on the plates were counted and an average count of each dilution was used to determine the appropriate amount of inoculum added to each sample.

Enrichment of samples: A variety of yoghurt samples (low-fat avocado, green lemon, kiwi fruit yoghurt, caramel yoghurt, strawberry and mulberry flavoured yoghurt, pure yoghurt with no sugar, and yoghurt-based drinks) were collected from a local supermarket. Labels on all samples indicated $>10^7$ CFU mL^{-1} of lactic acid bacteria. Samples were equilibrated to room temperature and 25 g samples were weighed into a sample enrichment bag (650 mL 3M Plain Sample bag, 3M Food Safety). In the initial trial, 225 mL of BPW ISO (3M Food Safety) was added to each of the samples (1:10 dilution) and inoculated with about 100-1000 CFU of *Salmonella* per sample. The samples were thoroughly mixed and incubated at 41.5°C for 30 hrs. Samples were tested by the LAMP assay at 24, 26, 28 and 30 hrs of incubation and culture-confirmed by GB 4789.4-2016 method at 30 hrs of incubation. In subsequent experiments, 25 g samples were tested with 1:20 dilution (475 mL of BPW ISO, 3M Food Safety). Several uninoculated and inoculated (about 3 and 78 CFU/25 g) samples were tested by the LAMP assay after incubation at 41.5°C for 24 hrs. Also, for some of the samples, *E. coli* at about 100 CFU/25 g was used as an interferent organism.

Lactic acid bacteria enumeration: The lactic acid bacteria count and the pH for each of the matrices was determined beforehand after enrichment. GB 4789.35-2016 requires enumeration of lactic acid bacteria on De Man Rogosa Sharpe (MRS) agar (anaerobic incubation) and *S. thermophilus* on Modified Chalmers (MC) agar (aerobic incubation)[26]. For lactic acid bacteria enumeration, samples from enrichment bags were serially diluted in Butterfield's buffer (3M Food Safety) and plated on 3M Petrifilm Lactic Acid Bacteria Count plates (3M Food Safety) and incubated at 36°C for 48 hrs. For *S. thermophilus* enumeration, serially diluted samples were plated on MC agar and incubated at 36°C for 72 hrs. According to GB 4789.35-2016, the lactic acid bacteria count includes a total of lactic acid bacteria counts from anaerobic incubation and *S. thermophilus* counts on MC agar[26].

***Salmonella* detection:** All enriched samples were tested with a *Salmonella* LAMP assay, MDA2SAL[21] (3M Food Safety). A 20 µL of the sample after enrichment was collected and processed for detection following manufacturer's instructions[21]. All primary enrichments were confirmed following GB 4789.4-2016 method[14]. The flow chart for the detection of *Salmonella* in yoghurt samples is shown in Fig. 1. All bacterial culture media for the GB method were

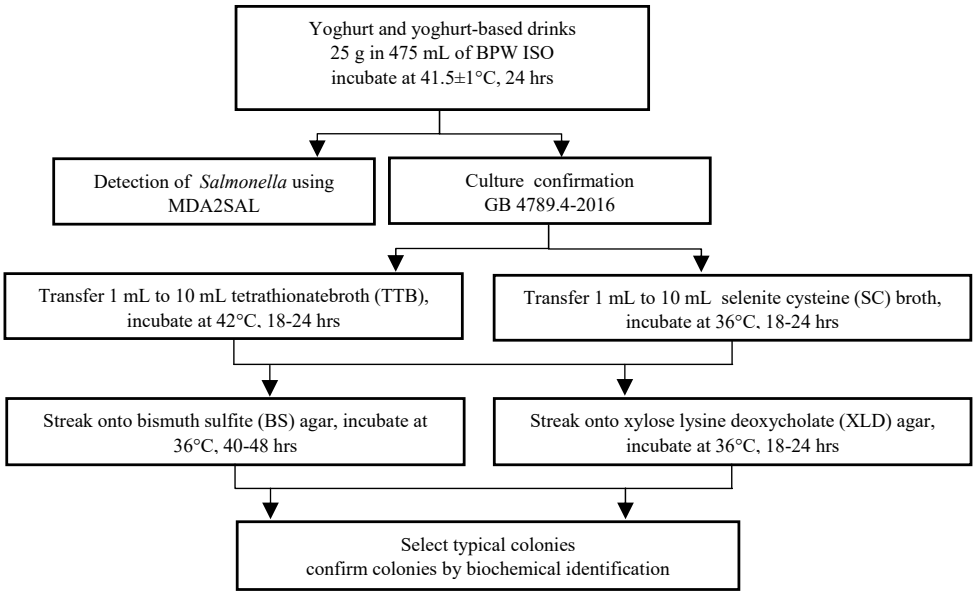

Fig. 1: Flow chart for detection of *Salmonella* in yoghurt samples by the LAMP method[21] and the GB 4789.4-2016 reference method[14]

obtained from Beijing Land Bridge Technology Co. Ltd. Biochemical confirmation of isolated colonies was done using API 20E strips (bio Mérieux China Limited, Beijing, China).

Analysis of results: Presumptive results obtained for *Salmonella* detection with the LAMP assay were compared with the culture-confirmed results. Probability of Detection (POD) was computed for the LAMP method (POD alternate, POD_a) and the culture confirmation by GB method (POD reference, POD_r) and used as a statistical model to compare the LAMP method to the GB method[27]. The difference between POD_a and POD_r, dPOD was computed and a 95% confidence interval for POD was calculated.

RESULTS AND DISCUSSION

In initial experiments, *Salmonella* failed to grow to detectable levels in 1:10 enrichments with the yoghurt samples tested even after 30 hrs of incubation at 41.5°C. Neither the LAMP assay nor the GB method was able to detect *Salmonella* with 1:10 enrichments in 16 out of 20 samples tested (Table 1). *Salmonella* was detected only in yoghurt-based drinks. *Salmonella* failed to grow in yoghurt samples even at high levels of artificial contamination (about 1000 CFU/25 g). The pH of BPW ISO was reduced to 4.2-4.3 after enrichment for all the yoghurt samples except yoghurt-based drinks which had a pH of 5.0 (Table 2). As the pH of yoghurt-based drinks was about 5.0, it probably allowed *Salmonella* growth.

In subsequent experiments, samples were tested with 1:20 dilution and *Salmonella* grew to detectable levels in 24 hrs of incubation at 41.5°C (Table 1). The pH of BPW ISO was reduced to 4.6-5.6 after enrichment for samples tested (Table 2). Samples did not show any natural contamination and inoculated samples were detected by the LAMP assay (Table 1). All the presumptive results were confirmed by the GB 4789.4-2016 culture method[14]. Based on these results, 1:20 dilution of samples enabled LAMP assay to provide next-day results for *Salmonella* detection. The presence of interferent organisms (*E. coli*) in *Salmonella* inoculated samples did not affect the detection of *Salmonella* and the LAMP assay did not detect *E. coli* in samples inoculated with *E. coli* alone.

The yoghurt samples had at least 10^7 CFU g^{-1} of lactic acid bacteria based on the label and the actual counts varied from 10^6-10^9 CFU g^{-1} (Table 2). The lactic acid bacteria count for the yoghurt samples did not change appreciably between 1:10 and 1:20 enrichments (Table 2). The initial pH of the matrices was in the range of 6.7-6.9. After enrichment for 24 hrs, the pH of 1:10 dilution samples was reduced to 4.2-4.3 indicating that the amount of BPW ISO was not enough to neutralize the pH to enable *Salmonella* growth. With 1:20 dilution, the pH of the enrichment after 24 hrs of incubation was 4.6-5.6 and *Salmonella* was able to grow at this level of pH. In additional experiments, vancomycin at 10 μg mL^{-1} was added to 1:10 enrichments to suppress lactic acid bacteria growth and this also enabled *Salmonella* growth (data not shown). However, 1:20 dilution is a better approach as it avoids the unnecessary use of antibiotics in testing.

Analysis of dPOD computed for the yoghurt samples (1:20 dilution) showed that the detection of *Salmonella* with the LAMP assay was not significantly different (95% confidence interval) from the GB culture confirmation method (Table 3).

LAMP uses a unique DNA polymerase for continuous DNA amplification that is resistant to matrix interference and inhibitors[15-19,21-25,28-31]. LAMP assays have been reported to have the same or higher sensitivity compared to PCR and culture-based assays in detecting foodborne pathogens such as *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, *Campylobacter*, from various food matrices[17,21-25,28-37].

Molecular methods based on the amplification of specific DNA targets in pathogenic microorganisms are more specific than the traditional methods that are 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 which allow next-day results as compared to 3-5 days for traditional testing[17,29,38-41].

The China food safety laws require accurate detection of foodborne pathogens in a variety of food samples. Yoghurt is considered fermented milk with zero tolerance for *Salmonella*[13]. The lactic acid bacteria in yoghurt, the low pH and antimicrobial compounds in yoghurt is thought to prevent the growth of foodborne pathogens. However, pathogens such as *Salmonella*, *E. coli* O157, *Listeria monocytogenes* have been shown to survive and grow in yoghurt samples[5-10]. A rapid method with optimized protocols for *Salmonella* detection in yoghurt samples will enable Chinese producers to assess the safety of these products quickly and release the product on time. In addition, the rapid method needs to be better or equivalent to the standard culture GB method. In this study, a *Salmonella* LAMP assay

Table 1: Detection of *Salmonella* with the 3M Molecular Detection Assay 2-*Salmonella* and the GB 4789.4-2016 method

Sample[a, b]	Number of samples	Presumptive result (MDA2SAL)[c]	Culture confirmed result[d]
1:10 enrichment			
Salmonella (about 87 CFU/25 g)[e]	20	4	4
Salmonella (about 78 CFU/25 g)	12	0	0
Salmonella (about 1000 CFU/25 g)	7	0	0
1:20 enrichment			
Uninoculated	13	0	0
Salmonella (about 3 CFU/25 g)	10	7	7
Salmonella (about 78 CFU/25 g)	17	17	17

[a]Samples included low-fat avocado, green lemon, kiwi fruit yoghurt, caramel yoghurt, strawberry and mulberry flavoured yoghurt, pure yoghurt with no sugar, and yoghurt-based drinks. The label on all samples indicated >10^7 CFU g^{-1} of lactic acid bacteria, [b]Twenty-five-gram samples were enriched in 225 mL (1:10) or 475 mL (1:20) BPW ISO and incubated at 41.5 °C for 24 hrs, [c]Number of positive samples detected by the LAMP assay, MDA2SAL[21], [d]Number of samples detected through culture. All samples were culturally confirmed regardless of presumptive results using GB 4789.4-2016 method[14] and [e]Samples were tested at 24, 26, 28 and 30 hrs of incubation by the LAMP assay and the results were similar at all time points

Table 2: pH and lactic acid bacteria count in yoghurt samples before and after enrichment in BPW ISO

	pH of 1:10 enrichment[a]		pH of 1:20 enrichment[a]		Lactic acid bacteria count[b] (log CFU g^{-1})			Modified Chalmers agar count[c] (log CFU g^{-1})		
Yoghurt sample	Before enrichment	After enrichment	Before enrichment	After enrichment	Before enrichment	After 1:10 enrichment	After 1:20 enrichment	Before enrichment	After 1:10 enrichment	After 1:20 enrichment
Low-fat avocado, green lemon, kiwi fruit yoghurt	6.7	4.2	6.9	4.6	6.6	7.2	6.5	9.0	6.5	8.1
Caramel yoghurt	6.8	4.2	6.9	4.9	5.7	5.5	5.3	8.9	6.0	5.8
Strawberry and mulberry flavored yoghurt	6.8	4.3	6.9	4.9	5.6	5.0	4.4	8.8	6.9	7.2
Pure yoghurt with no sugar	6.8	4.2	6.9	4.6	6.7	6.5	7.5	8.9	7.3	8.1
Yoghurt-based drinks	6.8	5.0	6.9	5.6	9.0	7.7	6.5	0.0	0.0	0.0

[a]Twenty-five-gram samples were enriched in 225 mL (1:10) or 475 mL (1:20) BPW ISO and incubated at 41.5 °C for 24 hrs, [b]Lactic acid bacteria count was determined using 3M Lactic Acid Bacteria Petrifilm Count plates and [c]MC agar was used per GB 4789.35-2016 to estimate the counts of *S. thermophilus*. According to GB 4789.35-2016, the total lactic acid bacteria count includes lactic acid bacteria counts under anaerobic conditions plus counts on MC agar[26]

Table 3: Probability of detection for the *Salmonella* LAMP assay and the GB 4789.4-2016 culture confirmation method for the detection of *Salmonella* in 1:20 BPW ISO enriched yoghurt samples

			Presumptive positives	Confirmed positives				95% CI[e]	
Matrix	Inoculation level	N[a]	(alternative)	(reference)	POD_a[b]	POD_r[c]	dPOD[d]	LCL	UCL
Yoghurt and yoghurt-based drinks	Uninoculated	13	0	0	0	0	0	-0.23	0.23
	Inoculated (about 3 CFU/25 g)	10	7	7	1	1	0	-0.36	0.36
	Inoculated (about 78 CFU/25 g)	17	17	17	1	1	0	-0.18	0.108

[a]N: Total number of samples analyzed, [b]POD_a: Probability of Detection for the alternative LAMP assay, [c]POD_r: Probability of Detection for the GB 4789.4-2016 culture reference method, [d]dPOD: Differential between the POD_a and the POD_r and [e]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

was compared to the culture confirmation by the GB method for the detection of *Salmonella* in yoghurt samples from China. The LAMP method with optimized protocols had equivalent sensitivity to the GB method. Also, the LAMP method provided next-day results compared to GB4789.4-2016 culture reference method requiring 3-5 days[14]. Hence, the LAMP method used in this study offered an easy-to-use analytical tool to assess the prevalence of *Salmonella* in yoghurt samples.

CONCLUSION

This study evaluated a *Salmonella* LAMP assay for rapid detection of *Salmonella* in yoghurt samples. Due to the low pH of enriched samples, *Salmonella* failed to grow to detectable levels under standard enrichment conditions (1:10 BPW ISO). However, with optimized growth conditions, the alternative LAMP assay enabled rapid detection of *Salmonella* in yoghurt and yoghurt-based drinks providing next-day results compared to 3-5 days for the GB method.

SIGNIFICANCE STATEMENTS

Salmonella is generally not considered a significant pathogen in fermented products. However, it can survive under low pH conditions and grow given appropriate conditions. Hence, dairy processors need effective control measures to assess contamination risks and rapid detection methods enable quicker action to ensure product safety. With the alternative LAMP assay, *Salmonella* can be detected next-day compared to 3-5 days for the culture-based GB method allowing dairy processors to quickly assess the safety of fermented products.

REFERENCES

1. Balasubramanian, R., J. Im, J.S. Lee, H.J. Jeon and O.D. Mogeni *et al*., 2019. The global burden and epidemiology of invasive non-typhoidal *Salmonella* infections. Hum. Vaccines Immunotherapeutics, 15: 1421-1426.
2. Chen, Y., W.X. Yan, Y.J. Zhou, S.Q. Zhen and R.H. Zhang *et al*., 2013. Burden of self-reported acute gastrointestinal illness in China: A population-based survey. BMC Public Health, Vol. 13. 10.1186/1471-2458-13-456.
3. Liu, X., Y. Chen, X. Wang and R. Ji, 2004. Foodborne disease outbreaks in China from 1992 to 2001 national foodborne disease surveillance system. Wei Sheng Yan Jiu, 33: 725-727.
4. Pei, X., N. Li, Y. Guo, X. Liu and L. Yan *et al*., 2015. Microbiological food safety surveillance in China. Int. J. Environ. Res. Public Health, 12: 10662-10670.
5. Álvarez-Ordóñez, A., L. Valdés, A. Bernardo, M. Prieto and M. López, 2013. Survival of acid adapted and non-acid adapted *Salmonella* Typhimurium in pasteurized orange juice and yogurt under different storage temperatures. Food Sci. Technol. Int., 19: 407-414.
6. Chang, H.S., D.H. Kim, D. Jeong, I.B. Kang and H.S. Kim *et al*., 2018. Fates of *Salmonella* Enteritidis and *Cronobacter sakazakii* in various multiple-strain yogurts and kefir during cold storage. J. Food Saf., Vol. 38. 10.1111/jfs.12429.
7. Cirone, K., Y. Huberman, C. Morsella, L. Méndez, M. Jorge and F. Paolicchi, 2013. Growth of *Mycobacterium avium* subsp. paratuberculosis, *Escherichia coli*, and *Salmonella* Enteritidis during preparation and storage of yogurt. Int. Scholarly Res. Notices, Vol. 2013. 10.1155/2013/247018.
8. Karagözlü, N., C. Karagözlü and B. Ergönül, 2007. Survival characteristics of *E. coli* O157:H7, *S. typhimurium* and *S. aureus* during kefir fermentation. Czech J. Food Sci., 25: 202-207.
9. Savran, D., F. Pérez-Rodríguez and A.K. Halkman, 2018. Modeling the survival of *Salmonella* Enteritidis and *Salmonella* Typhimurium during the fermentation of yogurt. Food Sci. Technol. Int., 24: 110-116.
10. Szczawiński, J., M.E. Szczawińska, A. Łobacz and A. Jackowska-Tracz, 2014. Modeling the effect of temperature on survival rate of *Salmonella* Enteritidis in yogurt. Pol. J. Vet. Sci., 17: 479-485.
11. Zhang, R. and J. Roberts, 2016. China's dairy import industry: An economic analysis of influencing trade factors. J. Manage. Sustainability, 6: 182-191.
12. Wang, Z., Y. Fan, Z. Zhang and S. Godefroy, 2017. Food Safety Standards. In: Food Safety in China: Science, Technology, Management and Regulation, Jen, J.J. and J. Chen (Eds.)., Wiley Online Library, pp: 363-380.
13. GGB 19302-2010, 2010. National Standard of the People`s Republic of China. National food safety standard - fermented milk. GB 19302-2010. Ministry of Health of the People's Republic of China, Beijing. http://tradechina.dairyaustralia.com.au/wp-content/uploads/2018/08/GB-19302-2010-National-Food-Safety-Standard-Fermented-Milk-f1-1-1.pdf.
14. GB 4789.4-2016, 2016. National Standard of the People's Republic of China. National food safety standard Food Microbiological Examination: *Salmonella*. http://tradechina.dairyaustralia.com.au/wp-content/uploads/2018/08/GB-4789.4-2016-Safety-Standard-Food-Microbiological-Examination-Salmonella.pdf

15. Mori, Y. and T. Notomi, 2009. Loop-mediated isothermal amplification (LAMP): A rapid, accurate and cost-effective diagnostic method for infectious diseases. J. Infect. Chemother., 15: 62-69.
16. Mori, Y., H. Kanda and T. Notomi, 2013. Loop-mediated isothermal amplification (LAMP): Recent progress in research and development. J. Infect. Chemother., 19: 404-411.
17. Niessen, L., J. Luo, C. Denschlag and R.F. Vogel, 2013. The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants. Food Microbiol., 36: 191-206.
18. Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, 2000. Loop-mediated isothermal amplification of DNA. Nucl. Acids Res., 28: e63-e63.
19. Notomi, T., Y. Mori, N. Tomita and H. Kanda, 2015. Loop-mediated isothermal amplification (LAMP): Principle, features, and future prospects. J. Microbiol., 53: 1-5.
20. Gandelman, O.A., V.L. Church, C.A. Moore, G. Kiddle and C.A. Carne *et al*., 2010. Novel bioluminescent quantitative detection of nucleic acid amplification in real-time. PLOS ONE, Vol. 5. 10.1371/journal.pone.0014155.
21. Bird, P., J. Flannery, E. Crowley, J.R. Agin and D. Goins *et al*., 2016. Evaluation of the 3M™ molecular detection assay (MDA) 2-*Salmonella* for the detection of *Salmonella* spp. in select foods and environmental surfaces: Collaborative study, first action 2016.01. J. AOAC Int., 99: 980-997.
22. Hu, L., L.M. Ma, S. Zheng, X. He and H. Wang *et al*., 2017. Evaluation of 3M molecular detection system and ANSR pathogen detection system for rapid detection of *Salmonella* from egg products. Poult. Sci., 96: 1410-1418.
23. Hu, L., X. Deng, E.W. Brown, T.S. Hammack, L.M. Ma and G. Zhang, 2018. Evaluation of Roka atlas *Salmonella* method for the detection of *Salmonella* in egg products in comparison with culture method, real-time PCR and isothermal amplification assays. Food Control, 94: 123-131.
24. Lim, H.S.Y., Q. Zheng, M. Miks-Krajnik, M. Turner and H.G. Yuk, 2015. Evaluation of commercial kit based on loop-mediated isothermal amplification for rapid detection of low levels of uninjured and injured *Salmonella* on duck meat, bean sprouts, and Fishballs in Singapore. J. Food Prot., 78: 1203-1207.
25. Yang, Q., K.J. Domesle, F. Wang and B. Ge, 2016. Rapid detection of *Salmonella* in food and feed by coupling loop-mediated isothermal amplification with bioluminescent assay in real-time. BMC Microbiol., Vol. 16. 10.1186/s12866-016-0730-7.
26. GB 4789.35-2010, 2010. National Food Safety Standard Food microbiological examination: Lactic acid bacteria. National Food Safety Standard of the People's Republic of China. https://www.svscr.cz/wp-content/files/zivocisne-produkty/GB4789._35_2010_food_microbiological_examination_lactic_acid_bacteria.pdf.
27. Wehling, P., R.A. LaBudde, S.L. Brunelle and M.T. Nelson, 2011. Probability of detection (POD) as a statistical model for the validation of qualitative methods. J. AOAC Int., 94: 335-347.
28. Rajagopal, R., C.A. Barnes, J.M. David, J. Goseland and J. Goseland, 2021. Evaluation of a commercial loop-mediated isothermal amplification assay, 3M™ molecular detection assay 2-campylobacter, for the detection of *Campylobacter* from poultry matrices. Br. Poult. Sci., 62: 404-413.
29. Yang, Q., K.J. Domesle and B. Ge, 2018. Loop-mediated isothermal amplification for *Salmonella* detection in food and feed: Current applications and future directions. Foodborne Pathog. Dis., 15: 309-331.
30. Yang, Q., F. Wang, W. Prinyawiwatkul and B. Ge, 2014. Robustness of *Salmonella* loop-mediated isothermal amplification assays for food applications. J. Appl. Microbiol., 116: 81-88.
31. Zhang, G., E.W. Brown and N. González-Escalona, 2011. Comparison of real-time PCR, reverse transcriptase real-time PCR, loop-mediated isothermal amplification, and the FDA conventional microbiological method for the detection of *Salmonella* spp. in produce. Appl. Environ. Microbiol., 77: 6495-6501.
32. Abatcha, M.G., P.L. Tan, L.O. Chuah, G. Rusul, S.R. Chandraprasad and M.E. Effarizah, 2020. Evaluation of 3M™ loop-mediated isothermal amplification-based kit and 3M™ ready-to-use plating system for detection of *Listeria* in naturally contaminated leafy vegetables, chicken, and their related processing environments. Food Sci. Biotechnol., 29: 1141-1148.
33. Bird, P., J. Flannery, E. Crowley, J. Agin and D. Goins *et al*., 2017. Evaluation of the 3M™ molecular detection assay (MDA) 2-*Listeria monocytogenes* for the detection of *Listeria monocytogenes* in a variety of foods and select environmental surfaces: Collaborative study, first action 2016.08. J. AOAC Int., 100: 454-469.
34. Bird, P., J. Flannery, E. Crowley, J. Agin and D. Goins *et al*., 2017. Evaluation of 3M molecular detection assay (MDA) 2–*Listeria* for the detection of *Listeria* species in select foods and environmental surfaces: Collaborative study, first action 2016.07. J. AOAC Int., 100: 82-98.
35. Domesle, K.J., Q. Yang, T.S. Hammack and B. Ge, 2018. Validation of a *Salmonella* loop-mediated isothermal amplification assay in animal food. Int. J . Food Microbiol., 264: 63-76.
36. Domesle, K.J., S.R. Young and B. Ge, 2020. Rapid screening for *Salmonella* in raw pet food by loop-mediated isothermal amplification. J. Food Prot., 84: 399-407.
37. Fortes, E.D., J. David, B. Koeritzer and M. Wiedmann, 2013. Validation of the 3M molecular detection system for the detection of *Listeria* in meat, seafood, dairy, and retail environments. J. Food Prot., 76: 874-878.

38. Jasson, V., L. Jacxsens, P. Luning, A. Rajkovic and M. Uyttendaele, 2010. Alternative microbial methods: An overview and selection criteria. Food Microbiol., 27: 710-730.
39. Mangal, M., S. Bansal, S.K. Sharma and R.K. Gupta, 2016. Molecular detection of foodborne pathogens: A rapid and accurate answer to food safety. Crit. Rev. Food Sci. Nutr., 56: 1568-1584.
40. Souii, A., M.B. M'hadheb-Gharbi and J. Gharbi, 2016. Nucleic acid-based biotechnologies for food-borne pathogen detection using routine time-intensive culture-based methods and fast molecular diagnostics. Food Sci. Biotechnol., 25: 11-20.
41. Wiedmann, M., S. Wang, L. Post and K. Nightingale, 2014. Assessment criteria and approaches for rapid detection methods to be used in the food industry. J. Food Prot., 77: 670-690.