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**Review Article**

# The Sensitivity and Specificity of Loop-Mediated Isothermal Amplification and PCR Methods in Detection of Foodborne Microorganisms: A Systematic Review and Meta-Analysis

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**Abstract**

**Background:** The loop-mediated isothermal amplification (LAMP) method is frequently used for identifying many microorganisms. The present review aimed to evaluate the sensitivity and specificity of LAMP method for detection of food-borne bacteria and to compare these features with those of polymerase chain reaction (PCR), as an alternative molecular diagnostic procedure, and with cultivation method, as the gold standard method.

**Methods:** The literature was searched in electronic databases (PubMed, Scopus, Web of Science, and EMBASE) for recruiting publications within Jan 2000 to Jul 2021. We used the combinations of keywords including foodborne disease, LAMP, PCR, Loop-mediated isothermal amplification, and polymerase chain reaction. Meta-analysis was used to adjust the correlation and heterogeneity between the studies. The efficiency of the methods was presented by negative likelihood ratio, positive likelihood ratio, sensitivity, specificity, and odds ratio using forest plots. A *P*-value less than 0.05 was considered as statistical significance cut off. The confidence intervals were presented at the 95% interval.

**Results:** Overall, 23 relevant studies were analyzed. The sensitivities of LAMP and PCR methods were estimated to be 96.6% (95% CI: 95.0-97.7) and 95.6% (95%CI: 91.5-97.8), respectively. The specificities of LAMP and PCR were also estimated to be 97.6% (95%CI: 92.6-99.3) and 98.7% (95%CI: 96.5-99.5), respectively.

**Conclusion:** The specificities of LAMP and PCR assays were determined by comparing their results with cultivation method as the gold standard. Overall, the specificity of both PCR and LAMP methods was low for detection of fastidious bacteria. Nevertheless, LAMP and PCR methods have acceptable specificities and sensitivities, and their application in clinical practice necessitates more studies.

**Keywords:** Food-borne pathogen; Specificity; Sensitivity; Loop-mediated isothermal amplification (LAMP); Polymerase chain reaction

## Introduction

In recent years, multiple molecular methods have been introduced for detecting different foodborne microorganisms. One of these methods is the loop-mediated isothermal amplification (LAMP) assay rapidly for rapid identification of a broad-range of microorganisms. In this assay, the amplification of the target sequence is carried out under isothermal temperature varying from 60 to 66 ºC (1). Similar to PCR, the LAMP assay also requires specific primers to amplify the target sequence. However, unlike PCR which needs one primer pair for amplification, the LAMP assay requires four or six specific primers (F3, B3, FIP, BIP, LB and LF) binding to six or eight separate regions within the target sequence (2). Consequently, the higher number of primers increases the efficiency and specificity of the assay (3). In the LAMP assay, the final product can be detected by the naked eye without any additional processing which is one of the advantages of LAMP assay (4). Despite many advantages, there are some argues regarding the specificity and sensitivity of LAMP assay.

Cultivation is considered as the gold standard method for detection of foodborne microorganisms growing in vitro (5). In fact, the specificity and sensitivity of other diagnostic methods are usually judged by culture results (6). There are multiple reports regarding the specificity and sensitivity of LAMP assay, and therefore, the current study aimed to compare the specificity and sensitivity of the LAMP assay with those of PCR and cultivation methods for detecting different foodborne microorganisms.

## Methods

The present meta-analysis was conducted to evaluate the sensitivity and specificity of two molecular techniques; LAMP and PCR and also to compare these specifications with those of the cultivation method as the gold standard.

Our study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (7).

### *Literature search*

The literature was searched in electronic databases (PubMed, Scopus, web of science, and EMBASE) within Jan 2000 to Jul 2021. In order to retrieve as many relevant studies as possible, different combinations of keywords including foodborne disease, LAMP, PCR, Loop-mediated isothermal amplification, and Polymerase chain reaction were utilized. Moreover, the reference lists of the relevant papers were scrutinized to include any missed studies (8).

### *Study selection*

Only full text English articles were included in the final analysis. At first, duplicate articles were removed. Then, the articles were screened by the titles and irrelevant ones were excluded. The abstracts of remaining articles were analyzed. Finally, those articles evaluating and comparing the three methods; LAMP and PCR and cultivation were selected. In order to be able to determine the sensitivities and specificities, the selected studies should have reported their results as false positive (FP), true positive (TP), false negative (FN) and true negative (TN). The microorganisms examined in the selected studies generally included naturally foodborne microorganisms. However, the food samples were artificially infected by with reference strains in some studies. The studies reporting the sensitivity and specificity indexes based on the CFU/ml or primer specificity were excluded from meta-analysis.

Finally, nine items were extracted from eligible articles, including author's name, the year of publication, country, studied microorganism, type of food sample, the total number of samples, utilized technique, and the rates of TP, TN, FN, FP, sensitivity and specificity.

### *Statistical analysis*

The data were analyzed using R version 3.4.1(9). The accuracy of the methods was presented as an overall negative likelihood ratio, positive likelihood ratio, sensitivity, specificity, and odds ratio. It was important to apply the same strategy to perform accurate analysis regarding sensitivities and specificities. For this, the random effect model of meta-analysis was used to adjust the correlation between sensitivities and specificities and also the heterogeneity between different studies. Due to the correlation between sensitivity and specificity, using the I-square statistic to estimate the level of heterogeneity was problematic. In other words, a large I-square statistic renders a high heterogeneity because of the correlation. The forest plot was used to estimate the overall negative likelihood ratio, positive likelihood ratio, sensitivity, specificity, and odds ratio. A *P*-value less than 0.05 was considered as the statistical significance cutoff. Confidence intervals were presented at the 95% level.

## Results

Overall, 16050 articles were retrieved from the initial search, of which 11419 were excluded as duplicates. Screening of the reminded articles by titles further omitted 3052 irrelevant studies. Totally, 672 studies were selected by screening the article abstracts, of which 248 were relevant by studying full texts (Fig. 1). Based on our selection criteria, 23 articles were finally analyzed (Table 1). Forest plots of the unadjusted results of these 23 studies have been shown in Figs. 2 and 3.

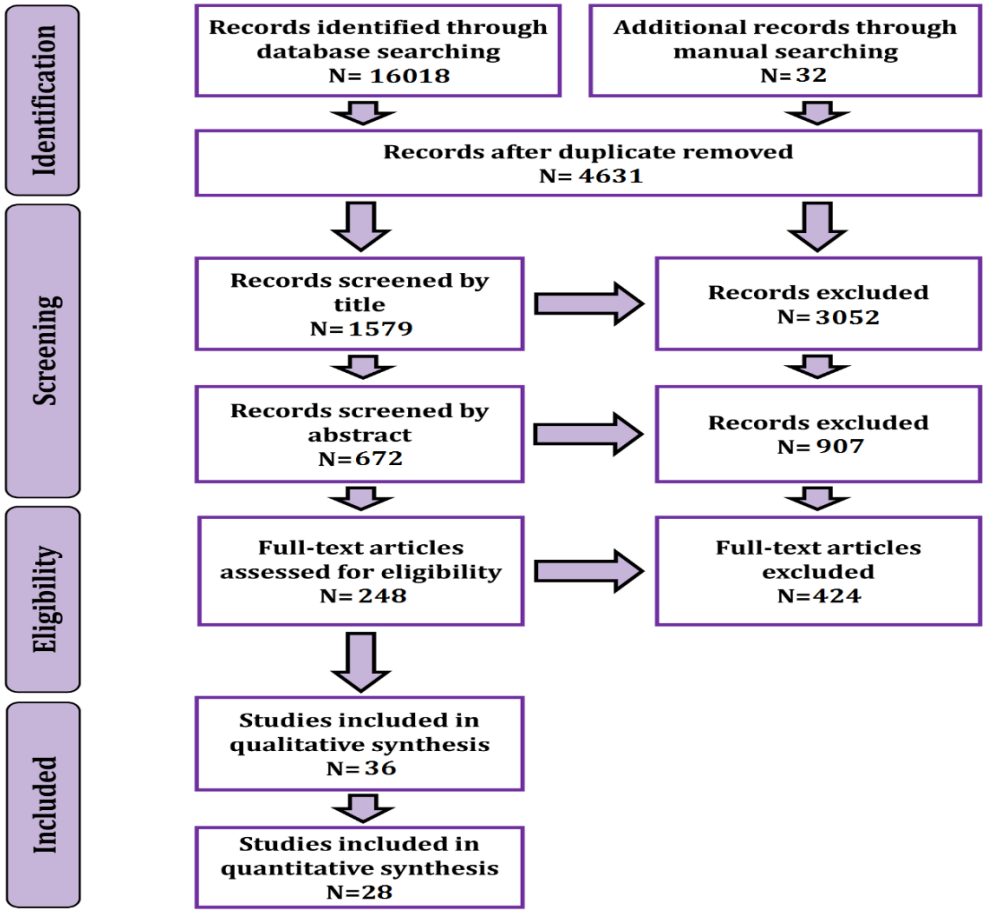


**Fig. 1:** The selection procedure for eligible studies to be included in the systematic review and meta-analysis

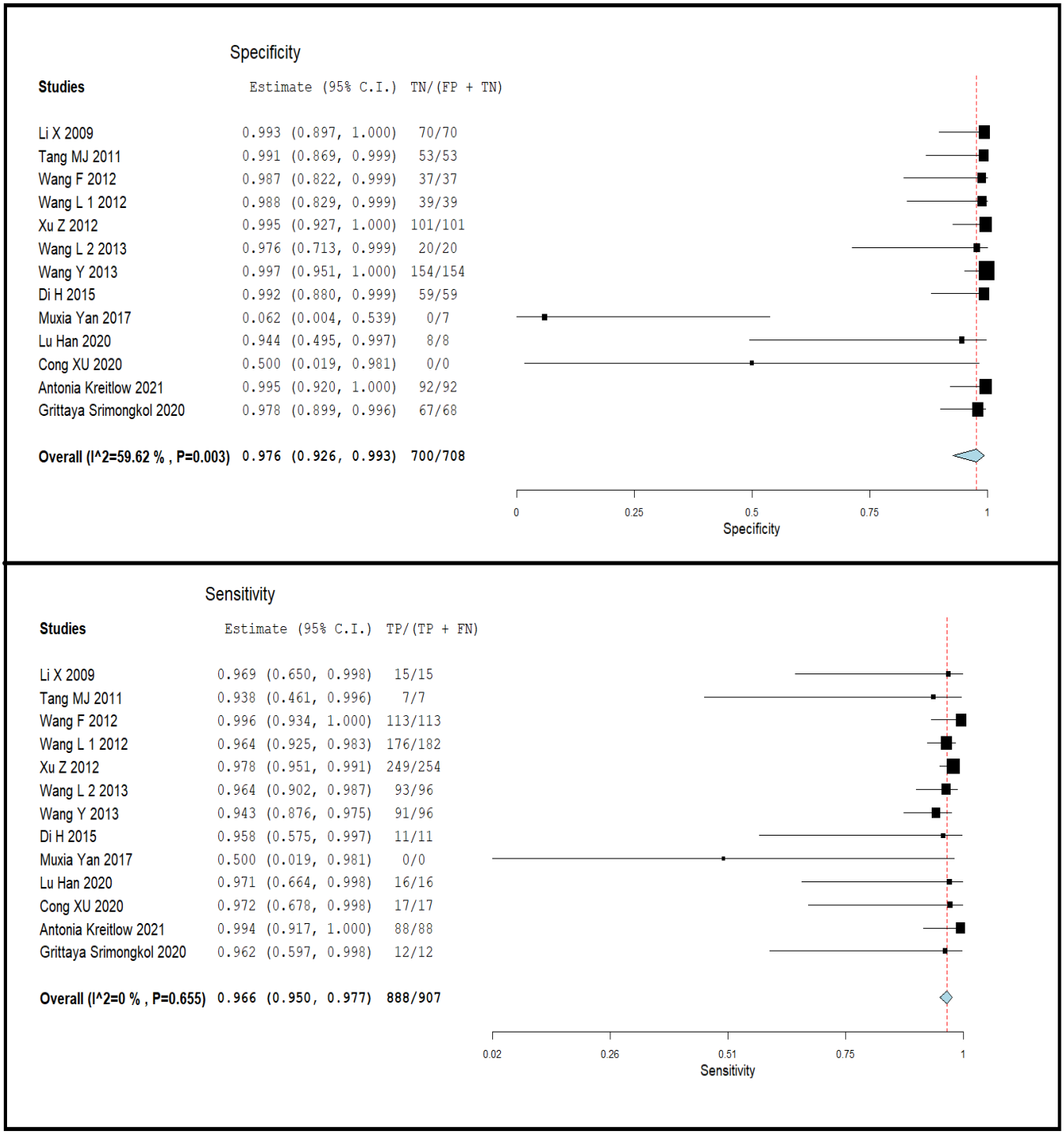


**Fig. 2:** The forest plots for estimating overall specificity (top chart) and sensitivity (bottom chart) of LAMP method. According to the included studies, the sensitivity and specificity were presented as 95 percent confidence intervals. The red vertical lines show either the overall sensitivity or specificity. The non-significant p-values of $I^2$ showed that there was no evidence of heterogeneity between the studies. According to the sample sizes of studies, the sizes of the black squares show the weight of each study. TN: true negative; FP: false positive

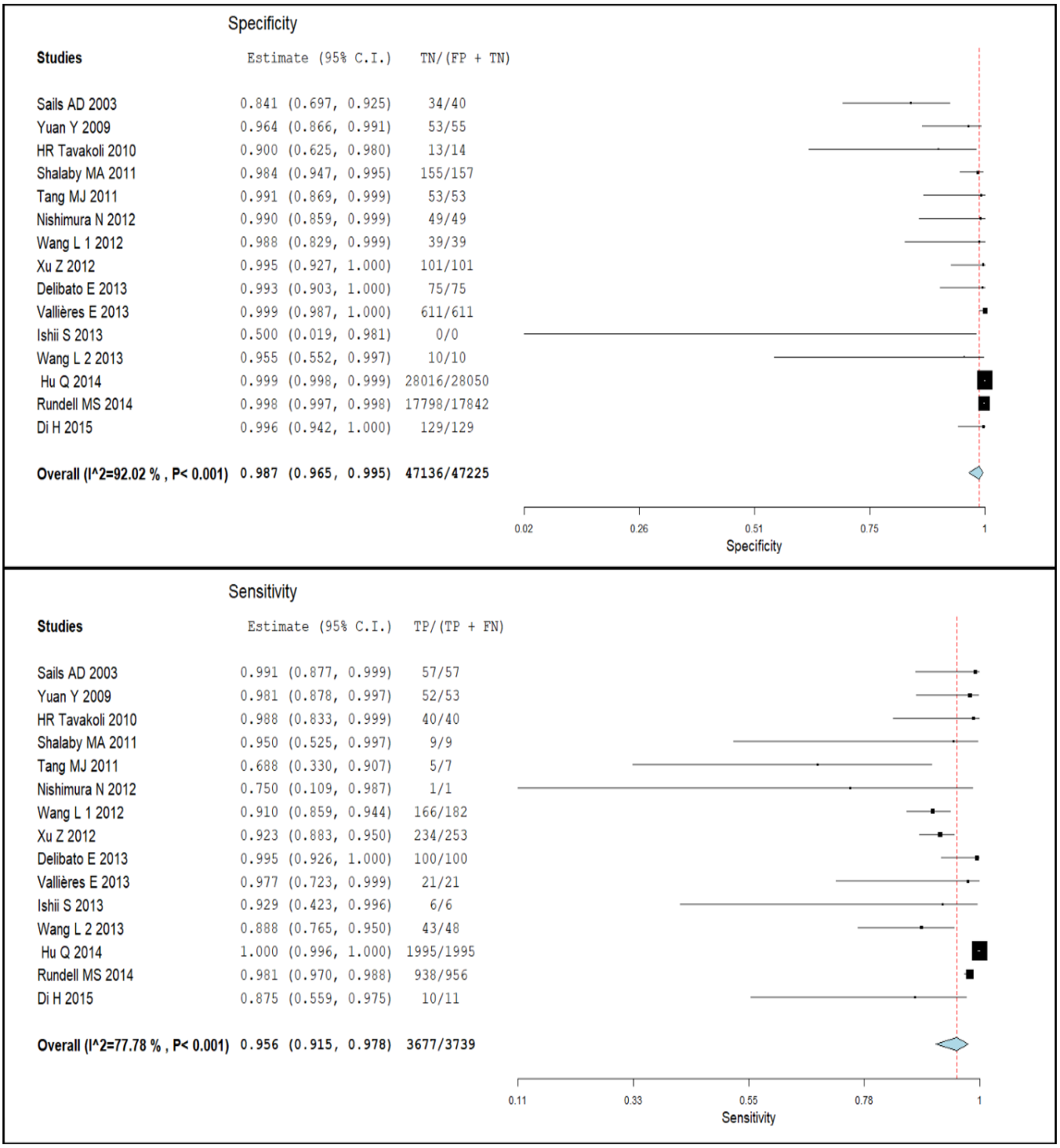


**Fig. 3:** The forest plots for estimating overall specificity (top chart) and sensitivity (bottom chart) of PCR. According to the included studies, the sensitivity and specificity were presented as 95 percent confidence intervals. The red vertical lines show either the overall sensitivity or specificity. The significant p-values of $I^2$ showed heterogeneity between the studies. According to sample sizes of the studies, the sizes of black squares show the weight of each study. TN: true negative; FP: false positive

**Table 1:** Studies included in meta-analysis for estimating the sensitivities and specificities of LAMP and PCR methods

| *Reference* | *Country* | *Microorganism* | *All samples* | *Food samples* | *Detection methods* | *Sensitivity* | *Specificity* | *Results* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | TP | FN | FP | TN |
| 17 | United Kingdom | *Campylobacter jejuni* | 97 samples | Raw poultry meat, offal, raw shellfish, and milk samples | qPCR | 100 | 85 | 57 | 0 | 6 | 34 |
| | | | | Raw poultry meat, offal, raw shellfish, and milk samples | | | | | | | |
| 18 | China | Salmonella strains Non-Salmonella strains | 85 samples | Minced meat of pig raw milk | LAMP | 100 | 100 | 15 | 0 | 0 | 70 |
| 19 | China | *Escherichia coli* | 36 samples | Eggs, raw sausage, salmon, ham, cooked ham, bacon, chicken, beef, pork, duck, hard cheese, raw-milk | Multiplex PCR | 100 | 80 | 52 | 1 | 2 | 53 |
| | | *Listeria monocytogenes* | | | | 100 | 100 | | | | |
| | | *Salmonella spp.* | | | | 92.30 | 95.65 | | | | |
| 20 | Iran | *Escherichia coli* | 18 samples | Eggs, raw milk, Raw Kobide, salad, chicken, cheese | Multiplex PCR | 100 | 100 | 40 | 0 | 1 | 13 |
| | | *Listeria monocytogenes* | | | | 100 | 100 | | | | |
| | | *Salmonella spp.* | | | | 100 | 80 | | | | |
| 21 | Egypt | *Listeria monocytogenes* | 66 100 | Clinical samples food samples | PCR | 100 | 98.72 | 9 | 0 | 2 | 155 |
| 22 | China | *Listeria monocytogenes* | 2 reference strains 10 target strain 10 non-listeria strains 60 chicken samples | Chicken samples | PCR | 71.42 | 100 | 5 | 2 | 0 | 53 |
| | | | | | LAMP | 100 | 100 | 7 | 0 | 0 | 53 |
| 23 | Louisiana, USA | Shiga toxin-producing *Escherichia coli* (STEC) | 50 STEC strains 40 non-STEC strains | Ground beef | Stx1-LAMP | 100 | 100 | 113 | 0 | 0 | 37 |
| | | | | | Stx2-LAMP | 100 | 100 | | | | |
| | | | | | Stx2-LAMP | 100 | 100 | | | | |
| 24 | Japan | Verotoxin-producing bacteria, *Salmonella, Shigella* | 50 Mixed human feces | NA* | PCR | 100 | 100 | 1 | 0 | 0 | 49 |
| 25 | China | *Listeria monocytogenes* | 182 Strains | Various food samples | LAMP | 96.70 | 100 | 176 | 6 | 0 | 39 |
| | | | | | PCR | 91.20 | 100 | 166 | 16 | 0 | 39 |
| 26 | USA | *Staphylococcus spp.* | 118 clinical isolates | NA | LAMP | 98 | 100 | 249 | 5 | 0 | 101 |
| | | | | | PCR | 92.49 | 100 | 234 | 19 | 0 | 101 |
| 27 | Italy | *Salmonella* | 175 samples (102 spiked samples and 73 real samples) | Minced meat and meat preparations made from poultry meat intended to be eaten cooked | qPCR | 100 | 100 | 100 | 0 | 0 | 75 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | Canada | Shiga toxin-producing *Escherichia coli* (STEC) | 632 stool samples from pediatric patients | NA | qPCR | 100 | 100 | 21 | 0 | 0 | 611 |
| 29 | Japan | *E. Coli serovars, Listeria monocytogenes serovars, Shigella spp. Salmonella spp. Vibrio cholerae, Campylobacter spp. Clostridium perfringens, Legionella spp.* | 6 Human fecal samples 40 Environmental water samples | NA | qPCR | 100 | | 6 | 0 | 0 | 0 |
| 30 | China | 48 *V. Parahaemolyticus* and 10 non- *V. Parahaemolyticus* strains | Seafood Samples | 20 fish, 10 shrimp, and 18 mussel samples and 10 non-*V. Parahaemolyticus* strains | PCR | 89.58 | 100 | 43 | 5 | 0 | 10 |
| | | | | | LAMP | 96.87 | 100 | 93 | 3 | 0 | 20 |
| 31 | China | *Salmonella enterica* | Artificial Contamination of Raw Milk | Raw milk | LAMP | 94.79 | 100 | 91 | 5 | 0 | 154 |
| 32 | China | *Salmonella enterica* subsp. Enterica *Listeria monocytogenes Escherichia coli* O157 *Vibrio parahaemolyticus V. Vulnificus Campylobacter jejuni Enterobacter sakazakii Shigella spp.* | Spiked stool samples | NA | Multiplex qPCR | 100 | 99.87 | 1995 | 0 | 34 | 28016 |
| 33 | USA | *C. Jejuni, V.fluvialis, V. Mimicus, V. Metschnikovii, V. Cholerae,* ETEC, *V. Parahaemolyticus, C. Coli V. Furnissii, EIEC, EPEC Y. Enterocolitica, DAEC Shigella spp Salmonella spp. S. Typhi L. Monocytogenes C. Lari STEC* | 97 stool and other clinical samples | NA | PCR | 98.11 | 99.75 | 938 | 18 | 44 | 17798 |
| 34 | China | *61 V. Parahaemolyticus* strains, 34 non-target strains | 70 seafood samples | All Samples | LAMP | 100 | 100 | 11 | 0 | 0 | 59 |
| | | | | Sleevefish, Oyster, Jellyfish, Weever, Shrimp, Tegillarca, Cuttlefish (n=10) | PCR | 90.90 | 100 | 10 | 1 | 0 | 129 |
| 35 | China | VBNC, Enterohemorrhagic *E. Coli* | Enterohemorrhagic *E. Coli* strain, ATCC43895 and 6 E. Coli strains | Various food samples during 2003-2007 | LAMP | 100 | 100 | 0 | 0 | 7 | 0 |
| 36 | Canada | 31 strains of both Gram-negative and Gram-positive bacteria (*Pseudomonas aeruginosa* ATCC 9721, *Listeria monocytogenes* , *Staphylococcus aureus* , *Campylobacter jejuni* ATCC 33560, *Campylobacter coli*) | Standard Strains | Samples of fresh produce | LAMP | 100 | 100 | 16 | 0 | 0 | 8 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 37 | China | *S. Aureus, Salmonella*, and *Shigella*, *L. Monocytogenes* | 17standard strains were used for specificity and sensitivity testing | Artificially contaminated juice | Multiplex LAMP | Sensitivity of mlamp was 10-fold higher than mpcr | 100 | 17 | 0 | 0 | 0 |
| 38 | Thailand | *Staphylococcus aureus* | 40 milk samples and 40 Pork samples | 40 ground pork and 40 milk samples | LAMP | 100 | 100 | 7 | 0 | 0 | 33 |
| | | | | | | 100 | 97 | 5 | 0 | 1 | 34 |
| 39 | Germany | *Salmonella* spp. | 180 bacterial Strains, 88 tested Salmonella strains, 92 tested non-Salmonella strains | RTE salad and Chicken carcass Minced meat Artificial contamination of food samples | LAMP | 100 | 100 | 88 | 0 | 0 | 92 |

*: Not Applicable, no food samples were evaluated in the study

Due to the correlation between the sensitivity and the specificity indexes, the data were analyzed using the DerSimonian and Laird methods. The random effects model was used to analyze the PCR data due to the high heterogeneity. Although there was no heterogeneity among LAMP data, the random effects model was also used to analyze the LAMP data for being able to compare its results with those of PCR. The sensitivity of LAMP and PCR method were estimated to be 96.6% (95% CI: 94.9%-97.7%) and 95.6% (95% CI: 91.5%-97.8%). The specificities of LAMP and PCR methods were also estimated to be 97.6% (95%CI: 92.6%- 99.3%) and 98.7% (95%CI: 96.5%-99.5%), respectively. Table 2 shows the sensitivities and specificities of LAMP and PCR methods in comparison with cultivation technique as the gold standard.

**Table 2:** Sensitivity and specificity of LAMP and PCR methods

| ***Variable*** | ***Model Results*** | | | | | | |
|---|---|---|---|---|---|---|---|
| | LAMP | | | | PCR | | |
| | Estimate | Lower bound | Upper bound | *P*-value | Estimate | Lower bound | Upper bound |
| Negative Likelihood Ratio | 0.048 | 0.016 | 0.146 | < 0.001 | 0.03 | 0.007 | 0.126 |
| Positive Likelihood Ratio | 39.176 | 12.423 | 123.548 | < 0.001 | 65.911 | 22.971 | 189.117 |
| Sensitivity | 0.966 | 0.950 | 0.977 | < 0.001 | 0.956 | 0.915 | 0.978 |
| Specificity | 0.976 | 0.926 | 0.993 | < 0.001 | 0.987 | 0.965 | 0.995 |
| Odds Ratio | 1409.797 | 327.498 | 6068.818 | < 0.001 | 2391.372 | 574.948 | 9946.395 |

*P* value= <0.001

## Discussion

LAMP and PCR are two molecular methods frequently used to identify microorganisms in research and clinical settings. There are many studies indicating that LAMP assay benefits from higher sensitivity and specificity in comparison with other molecular detection methods such as PCR and Real-time PCR (10, 11). In the present meta-analysis, we evaluated the sensitivities and

specificities of LAMP and PCR techniques in detection of foodborne transmitted bacteria and compared them to those of culture technique as the gold standard. To the best of our knowledge, this is the first comprehensive systematic review and meta-analysis estimating the sensitivities and specificities of LAMP and PCR methods for detecting foodborne bacteria.

Cultivation is considered as the gold standard method for detection of foodborne pathogens. However, several alternative molecular assays have recently been introduced that are user friendly and easy to perform. LAMP and PCR techniques are two common for detecting foodborne pathogens in food and stool specimens (12).

According to our statistical analysis, sensitivities of LAMP and PCR techniques were estimated to be 96.6% and 95.6% ($P$<0.001), respectively. Since the low initial copies of pathogens in food specimens may be lost during sample processing, evaluating sensitivity is an important factor for diagnostic methods of microorganisms. Rapid detection methods usually have high sensitivities. In fact, molecular methods are considered to be highly sensitive in comparison with conventional procedures due to their short-term running period. Rapid methods such as PCR and LAMP reduce user-born errors during the experiment rendering them more sensitive than the methods with long processing periods (13). Considering the fact that many factors could kill alive bacteria, the bacterial count is usually low in stool specimens. Therefore, the methods with high sensitivities are more useful and reliable in these conditions. We here observed that the sensitivity of PCR was slightly higher than LAMP rendering PCR as a valuable diagnostic method in these conditions.

The larger number of primers per target in LAMP increases the primer-primer interactions. The LAMP product is a series of concatemers of the target region, giving rise to a characteristic "ladder" or banding pattern on a gel, rather than a single band as with PCR and it seems to be less sensitive than PCR to inhibitor in case of complex samples, likely due to the use of a DNA polymerase rather than *Taq* polymerase as in PCR.

The specificity of a diagnostic test refers to the accuracy of the test in diagnosis of true negative cases. Therefore, a test with high specificity should render negative results in germ-free specimens. In the present study, the specificities of LAMP and PCR methods were estimated to be 97.6% and 98.7% ($P$<0.001) respectively. In LAMP method, the target gene is amplified using four pairs of primers improving the reaction specificity. In other words, using additional specific primers reduces the rate of false positive results (14). There are also many publications indicating a higher specificity for LAMP method than other diagnostic tests (15, 16).

The specificity of LAMP and PCR procedures is usually determined by comparing the results with the cultivation method as the gold standard. For fastidious microorganisms that barely grow on commercial media, the specificity of molecular methods will decrease because of the exaggerated false positive results. Therefore, it is best to consider the specificity of molecular methods in regard to the target microorganisms.

## Conclusion

The LAMP and PCR methods have acceptable specificities and sensitivities necessitating conduction of more studies to establish them as routine and valid diagnostic modalities.

## Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

## Financial source

The author declares that there was no financial support for this study.

## Conflict of interest

The authors declare that there is no conflict of interests.

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