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 °C (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 I² 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

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Microorganism</th>
<th>All samples</th>
<th>Food samples</th>
<th>Detection methods</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>United Kingdom</td>
<td>Campylobacter jejuni</td>
<td>97 samples</td>
<td>Raw poultry meat, offal, raw shellfish, and milk samples&lt;br&gt;Raw poultry meat, offal, raw shellfish, and milk samples</td>
<td>qPCR</td>
<td>100</td>
<td>85</td>
<td>5 0 6 34</td>
</tr>
<tr>
<td>18</td>
<td>China</td>
<td>Salmonella strains&lt;br&gt;Non-Salmonella strains</td>
<td>85 samples</td>
<td>Minced meat of pig raw milk</td>
<td>LAMP</td>
<td>100</td>
<td>100</td>
<td>1 0 0 70</td>
</tr>
<tr>
<td>19</td>
<td>China</td>
<td>Escherichia coli&lt;br&gt;Listeria monocytogenes&lt;br&gt;Salmonella spp.</td>
<td>36 samples</td>
<td>Eggs, raw sausage, salmon, ham, cooked ham, bacon, chicken, beef, pork, duck, hard cheese, raw-milk</td>
<td>Multiplex PCR &amp; LAMP</td>
<td>100</td>
<td>80</td>
<td>5 1 2 53</td>
</tr>
<tr>
<td>20</td>
<td>Iran</td>
<td>Escherichia coli&lt;br&gt;Listeria monocytogenes&lt;br&gt;Salmonella spp.</td>
<td>18 samples</td>
<td>Eggs, raw milk, Raw Kobilde, salad, chicken, cheese</td>
<td>Multiplex PCR</td>
<td>100</td>
<td>100</td>
<td>4 0 1 13</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Egypt</td>
<td>Listeria monocytogenes&lt;br&gt;Salmonella spp.</td>
<td>66&lt;br&gt;100</td>
<td>Clinical samples&lt;br&gt;Minced meat and meat preparations made from poultry meat intended to be eaten cooked</td>
<td>PCR</td>
<td>100</td>
<td>98.72</td>
<td>9 0 2 15</td>
</tr>
<tr>
<td>22</td>
<td>China</td>
<td>Listeria monocytogenes</td>
<td>2 reference strains&lt;br&gt;2 target strain&lt;br&gt;10 target strain&lt;br&gt;60 chicken samples</td>
<td>Chicken samples</td>
<td>PCR</td>
<td>71.42</td>
<td>100</td>
<td>5 2 0 53</td>
</tr>
<tr>
<td>23</td>
<td>Louisiana, USA</td>
<td>Shiga toxin-producing Escherichia coli (STEC)</td>
<td>50 STEC strains&lt;br&gt;40 non-STEC strains</td>
<td>Ground beef</td>
<td>LAMP&lt;br&gt;Sxt1-LAMP&lt;br&gt;Sxt2-LAMP&lt;br&gt;Sxt2-LAMP</td>
<td>100&lt;br&gt;100&lt;br&gt;100&lt;br&gt;100</td>
<td>100</td>
<td>7 0 0 53</td>
</tr>
<tr>
<td>24</td>
<td>Japan</td>
<td>Verotoxin-producing bacteria, Salmonella, Shigella</td>
<td>50 Mixed human feces</td>
<td>NA*</td>
<td>PCR</td>
<td>100</td>
<td>100</td>
<td>1 0 0 49</td>
</tr>
<tr>
<td>25</td>
<td>China</td>
<td>Listeria monocytogenes&lt;br&gt;Staphylococcus spp.</td>
<td>182 Strains</td>
<td>Various food samples</td>
<td>LAMP&lt;br&gt;PCR</td>
<td>96.70&lt;br&gt;91.20</td>
<td>100</td>
<td>6 0 39</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>26</td>
<td>USA</td>
<td>Staphylococcus spp.</td>
<td>118 clinical isolates</td>
<td>NA</td>
<td>LAMP&lt;br&gt;PCR</td>
<td>98&lt;br&gt;92.49</td>
<td>100</td>
<td>2 5 0 10</td>
</tr>
<tr>
<td>27</td>
<td>Italy</td>
<td>Salmonella</td>
<td>175 samples&lt;br&gt;(102 spiked samples and 73 real samples)</td>
<td>Minced meat and meat preparations made from poultry meat intended to be eaten cooked</td>
<td>qPCR</td>
<td>100</td>
<td>100</td>
<td>1 0 0 75</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
<th>Country</th>
<th>Description</th>
<th>Sample Type</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>LOD</th>
</tr>
</thead>
</table>
| 28 | Canada  | Shiga toxin-producing 
*Escherichia coli* (STEC) | 632 stool samples from pediatric patients | qPCR | 100 | 100 | 2 | 0 | 0.61 |
| 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 | qPCR | 100 | 100 | 1 | 0 | 0 |
| 30 | China   | 48 *V. Parahaemolyticus* and 10 non-*V. Parahaemolyticus* strains | Seafood Samples | PCR | 89.58 | 100 | 4 | 5 | 0.10 |
|     |         |             |             | LAMP | 96.87 | 100 | 9 | 3 | 0.20 |
| 31 | China   | *Salmonella enterica* subsp. Enterica 
*Listeria monocytogenes* 
*Escherichia coli* O157 
*Vibrio parahaemolyticus* 
*V. Vulnificus* 
*Campylobacter jejuni* 
*Enterobacter sakazakii* 
*Shigella* spp. 
*ETEC, V. Parahaemolyticus, C. Coli 
V. Farnissii, EIEC, EPEC, Y. Enterocolitica, 
DAEC, Shigella spp, 
Salmonella spp. 
*S. Typhi* 
*L. Monocytogenes* 
C. Lari 
STEC | Artificial Contamination of Raw Milk | qPCR | 99.87 | 100 | 9 | 1 | 0.15 |
| 32 | China   | *Salmonella enterica* subsp. Enterica 
*Listeria monocytogenes* 
*Escherichia coli* O157 
*Vibrio parahaemolyticus* 
*V. Vulnificus* 
*Campylobacter jejuni* 
*Enterobacter sakazakii* 
*Shigella* spp. 
*ETEC, V. Parahaemolyticus, C. Coli 
V. Farnissii, EIEC, EPEC, Y. Enterocolitica, 
DAEC, Shigella spp, 
Salmonella spp. 
*S. Typhi* 
*L. Monocytogenes* 
C. Lari 
STEC | Spiked stool samples | Multiplex qPCR | 99.87 | 100 | 9 | 1 | 0.28 |
| 33 | USA     | *C. jejuni*, V. fluvialis, V. Mimmicus, V. Metchnikovi, V. Cholerae, 
*ETEC, V. Parahaemolyticus, C. Coli 
V. Farnissii, EIEC, EPEC, Y. Enterocolitica, 
DAEC, Shigella spp, 
Salmonella spp. 
*S. Typhi* 
*L. Monocytogenes* 
C. Lari 
STEC | 97 stool and other clinical samples | PCR | 98.11 | 99.75 | 9 | 1 | 0.17 |
|     |         |             |             | LAMP | 100 | 100 | 1 | 8 | 0.79 |
| 34 | China   | 61 *V. Parahaemolyticus* strains, 34 non-target strains | 70 seafood samples | LAMP | 100 | 100 | 1 | 0 | 0.59 |
|     |         |             |             | PCR | 90.90 | 100 | 1 | 0 | 0.12 |
|     |         |             |             | LAMP | 100 | 100 | 1 | 0 | 0.12 |
| 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 | 0.07 |
| 36 | Canada  | 31 strains of both 
Gram-negative and 
Gram-positive bacteria (Pseudomonas aeruginosa ATCC 9721, 
*Listeria monocytogenes*, 
*Staphylococcus aureus*, 
*Campylobacter jejuni* 
ATCC 33560, *Campylo-
bacter coli*) | Standard Strains | LAMP | 100 | 100 | 1 | 0 | 0.08 |
Sadeghi et al.: The Sensitivity and Specificity of Loop-Mediated Isothermal Amplification ...

37 China Staphylococcus aureus, Salmonella, and Shigella, L. Monocytogenes 17 standard strains were used for specificity and sensitivity testing Artificially contaminated juice Multiplex LAMP Sensitivity of mlam p was 10-fold higher than mpcr 100 1 0 0

38 Thailand Staphylococcus aureus 40 milk samples and 40 Pork samples 40 ground pork and 40 milk samples LAMP 100 1 0 0 33

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 1 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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAMP</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>Negative Likelihood Ratio</td>
<td>0.048</td>
</tr>
<tr>
<td>Positive Likelihood Ratio</td>
<td>39.176</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.966</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.976</td>
</tr>
<tr>
<td>Odds Ratio</td>
<td>1409.797</td>
</tr>
</tbody>
</table>

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

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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 de-
tecting 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 food-
borne 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 peri-
od. Rapid methods such as PCR and LAMP re-
duce user-born errors during the experiment ren-
dering 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 speci-
mens. Therefore, the methods with high sensitivi-
ities are more useful and reliable in these condi-
tions. 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 com-
plex samples, likely due to the use of a DNA pol-
imerase 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 spec-
imens. 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 spe-
cific primers reduces the rate of false positive re-
sults (14). There are also many publications indi-
cating 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 con-
sider the specificity of molecular methods in re-
gard to the target microorganisms.

Conclusion

The LAMP and PCR methods have acceptable
specificities and sensitivities necessitating con-
duction of more studies to establish them as rou-
tine and valid diagnostic modalities.

Ethical considerations

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

Financial source

The author declares that there was no financial
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Conflict of interest

The authors declare that there is no conflict of interests.

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