Journal of Microbiological Methods 165 (2019) 105697

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

# Validation and verification of LAMP, ISO, and VIDAS UP methods for detection of *Escherichia coli* O157:H7 in different food matrices

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ARTICLE INFO

*Keywords:*
*E. coli* O157:H7
LAMP
Specificity
Sensitivity

ABSTRACT

*Escherichia coli* O157:H7 is one of the most important food-borne pathogens to threaten public health. Cultural methods are used as a gold standard while they are laborious and time-consuming. Loop-mediated isothermal amplification (LAMP) method is an alternative method that became widely used for the detection of food-borne pathogens. The aim of this study was to evaluate the specificity and sensitivity of LAMP method for detection of *E. coli* O157:H7, also to compare detection performances with VIDAS UP and ISO (International Organization for Standardization) methods in different food matrices (beef meat, minced lamb meat, milk, cheese, apple puree, and soybean sprouts). *E. coli* O157:H7 were spiked in three different levels (high 4.58; medium 2.32; low 0.30 $\log_{10}$ CFU/g-ml) to food matrices. Although there were no significant differences in terms of the specificity and sensitivity values among the three methods ($p \geq .05$), it was determined that the highest specificity and sensitivity values obtained from the LAMP method. Sensitivity and specificity values of LAMP method were found as 0.997 and 0.988, for the ISO method were 0.989 and 0.971, and for the VIDAS UP method were 0.980 and 0.963, respectively. In milk samples, sensitivity and specificity values of the VIDAS UP method were significantly lower than LAMP and ISO methods ($p < .05$). However, there were no significant differences found for the other food matrices among the three methods ($p > .05$). It can be summarized from this study that specificity and sensitivity values of the LAMP method are equal or higher and less time-consuming than ISO and VIDAS UP methods. In conclusion, using a simple, fast, and inexpensive detection method, such as LAMP, especially in endemic regions or in an outbreak to control spreading of pathogens, is very important for public health.

## 1. Introduction

Food-borne pathogens found in food and water samples are lower in counts (Aminul Islam et al., 2006). Sensitive, reliable and fast detection methods are required for ensuring the food safety (Conrad et al., 2014; Wang et al., 2017). Although, the conventional culture methods are gold standard and accepted as irreplaceable, they are time consuming and laborious (Mori and Notomi, 2009; Gehring et al., 2006; Niessen et al., 2013; Zhao et al., 2010). Also, culture-based detection methods require enrichment step on selective agar media and followed by confirmation which include biochemical, serological or molecular methods (Kumar and Mondal, 2015). For this purpose, rapid detection methods have been developed and which are divided into immunological, nucleic acid amplification, and biosensor-based methods (Law et al., 2015).

Shiga toxin-producing *Escherichia coli* (STEC) strains predominant serotype O157 is one of the most important food-borne pathogens, and threat to public health globally (Hara-Kudo et al., 2008; Aminul Islam et al., 2006; Carvalho et al., 2014). This pathogen is responsible for hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans (Oporto et al., 2008) and the minimum infectious dose to humans is < 10 pathogenic bacteria (Ngwa et al., 2013; Zhang et al., 2016; Quilliam et al., 2011). The gastrointestinal tract of cattles are the main reservoir of this pathogen (Carvalho et al., 2014) and some food such as milk and milk products, meat and meat products, vegetables, and fruits are closely related to outbreaks (Zhao et al., 2013; Sidari and Caridi, 2011). Annually, STEC strains causes approximately 176,000 cases, 2400 hospitalizations, and 20 deaths in United States (Scallan et al., 2011). Therefore, rapid, sensitive, cheap and reliable methods are needed to detect this bacterium (Zhao et al., 2010; Hara-Kudo et al., 2007).

Researchers have developed methods to detect STEC strains

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https://doi.org/10.1016/j.mimet.2019.105697
Received 1 July 2019; Received in revised form 13 August 2019; Accepted 20 August 2019
Available online 21 August 2019

serogroup O157 by using selective cultuvation, immunochromatographic, immunomagnetic separation or immunological confirmation tests (Jung et al., 2005; O'Brien et al., 2005). In addition, nucleic acid amplification method, termed loop-mediated isothermal amplification (LAMP) has been developed by Notomi et al. (2000). This method amplifies the target DNA with high accuracy, efficiency and quickly under isothermal conditions (60–65 °C), which depends on autocycling strand displacement DNA synthesis carried out by Bst DNA polymerase with four to six specific primers (Wang et al., 2017; Maruyama et al., 2003; Chen et al., 2011; Sasitharan et al., 2013). LAMP method has some advantages and differences from other methods. First of all, this method does not require specialized and expensive equipments (thermal cycler and gel electophoresis) (Yano et al., 2007; Quilliam et al., 2011). Second, it is highly specific due to use of 4 or 6 different primers which carry out the amplification of the target DNA (Jiang et al., 2012). Finally, it is faster and easier to apply than PCR (Kouguchi et al., 2010), and in a short time large amounts of target DNA can be synthesized, positive results can be detected visibly without using any sophisticated equipments (Wang et al., 2012a). Some organic PCR inhibitors showing inhibitory effect in the PCR reported to have no effect on efficiency of LAMP (Kumar and Mondal, 2015). Therefore, LAMP method could become a useful instrument for the rapid detection of pathogen microorganisms which are important for food safety and public health.

Development of a fast, accurate and sensitive method for the detection of pathogen microorganisms is fundamental to control foodborne diseases and outbreaks. Therefore, the aim of this study was to not only evaluate the specificity and sensitivity of LAMP method for detection of *E. coli* O157:H7, but also to compare detection perfomances with ISO 16654 (International Organization for Standardization-ISO 16654, 2003) and VIDAS UP methods in different food matrices.

## 2. Materials and methods

### 2.1. Materials

Six different food matrices including beef cuts, minced lamb meat (subcutaneous fat trimmed *musculus longissimus dorsi* was used in meat samples which contain ~3% intramuscular fat) whole milk (at least 3.5% fat), full-fat white cheese (at least 45% fat in dry matter), apple puree, and soybean sprouts used in the study were obtained from a local supermarket. Food samples were analyzed for *E. coli* O157:H7 according to standard analysis protocol (International Organization for Standardization-ISO 16654, 2003) before used in the study, and *E. coli* O157:H7 negative food samples were used for the experiment.

#### 2.1.1. E. coli O157:H7 strains and inoculum preperation

In this study, three reference and 5 wild type *E. coli* O157:H7 strains were used. Reference strains were ATCC 43985, ATCC 43984, and ATCC 35150. Wild type strains ECO157–1, ECO157–2 and ECO157–3 were isolated from meat, ECO157–4 isolated from salad and ECO157–5 isolated from beef steak. All strains were incubated in Tyriptic Soy Broth (Merck, Darmstad, Germany) at 37 °C for 24 h to obtain fresh culture. At the end of the incubation period, the broths were centrifuged at 4,000 rpm for 10 min. Then supernatant was removed and the pellets were dissolved in 1 ml 0.1% Pepton Water (Merck, Darmstad, Germany) and strains mixed to obtain a bacterial cocktail and final volume was adjusted to 10 ml with PW. Bacterial cocktail was diluted with 0.1% PW to reach each of the spike levels used in the study. Subsequently, 0.25 ml inoculum from each of the spike level, directly added to the 25 ml liquid food samples and 0.25 ml inoculum spreaded to the surfaces of solid food samples and sprayed onto the soybean sprouts. After inoculation, at least 10 min was given to the cultures for the bacterial attachment to the samples. Three spike levels used in the study were as follows, high (4.58 $\log_{10}$ CFU/g-ml), medium (2.32 $\log_{10}$ CFU/g-ml) and low (0.30 $\log_{10}$ CFU/g-ml). For the negative control, analyzes were performed without any contamination to the food samples that were already analyzed found negative for *E. coli* O157:H7 analysis. In the study, different food matrices (6) x subsamples (10) x spike levels (3) x replicate (2), totally 360 samples were used for positive control samples, and different food matrices (6) x subsamples (20) x replicate (2), totally 240 negative control samples were used for each of the three detection methods.

### 2.2. Methods

#### 2.2.1. Lamp assay

25 g/mL samples were taken from spiked food samples into the sterile sampling bag under aseptic conditions, then 225 ml modified Tyriptic Soy Broth with novobiocin (mTSB + N) (Merck, Darmstadt, Germany) was added and incubated at 41.5 ± 1 °C for 18–24 h. At the end of the incubation period, 50 µl of enrichment broth was used in LAMP analysis. Verotoxin-producing *Escherichia coli* detection kit (Eiken Chemical, Tokyo, Japan) were used according to the manufacturer's instruction. For DNA extraction 50 µl of the enrichment broth was mixed with 50 µl of EXF (including in the kit) and incubated at 95 °C for 5 min. This mixed solution was centrifuged for 1 min at 2000 × *g*. After centrifuge, 5 µl of the supernatant was taken and added to the reaction tube with 20 µl of reaction mixture. The reaction was carried out in water bath at 65 °C for 60 min. Posivite reactions in tubes were detected by visually.

#### 2.2.2. ISO-16649 assay

25 g/mL samples were taken from spiked food samples into the sterile sampling bag under aseptic conditions and 225 ml modified Tyriptic Soy Broth with novobiocin (mTSB + N) (Merck, Darmstadt, Germany) added. Subsequently, pre-enrichment culture incubated at 41.5 ± 1 °C for 18–24 h. After pre-enrichment step, immunomagnetic separation was carried out according to the manufacturer's instruction by using the Dynabeads anti-*E. coli* O157 (71,004, Invitrogen, Dynal, Norway). After immunomagnetic separation, 50 µl of Dynabead and bacterial complexes were added on Sorbitol MacConkey agar (CT-SMAC) (Merck, Darmstadt, Germany) containing Cefixime Tellurite and plates were incubated at 37 °C for 18–24 h. At the end of the incubation period, non-fermentative colonies in petri plates were confirmed using latex agglutination test (DR0620, Oxoid, UK).

#### 2.2.3. VIDAS UP assay

25 g/mL samples were taken from spiked food samples into the sterile sampling bag, then 225 mL buffered pepton water added and homogenized. Subsequently, pre-enrichmet culture incubated at 41.5 ± 1 °C for 24 h. After the pre-enrichment step, 0.5 ml of samples were added to the VIDAS UP test kits wells. Then kits were heated on the VIDAS UP HEAT&GO (BioMérieux, France) for 5 min and VIDAS UP analysis were made according to the manufacturer's instruction, subsequently the results were evaluated.

### 2.3. Statistical analysis

Chi-squared Fisher's exact test was performed for comparison of three diffrent methods. Statistical significance level was accepted as $p < .05$. All statistical analyzes were performed by using SPSS 21.0 package program (SPSS, 2012).

## 3. Results

Sensitivity and specificity values of the three methods are given in Table 1. Sensitivity and specificity values of LAMP method were found 0.997 and 0.988, respectively. Sensitivity and specificity values of the ISO method were 0.989 and 0.971, and for the VIDAS UP method were found as 0.980 and 0.963, respectively. Although specificity and

**Table 1**
Sensitivity and specificity values of LAMP, ISO, and VIDAS UP methods detection of *Escherichia coli* O157:H7 (n: 360 for positive control, n: 240 for negative control).

| Method | Recovery results (%) | | | | Sensitivity[c] | Specificity[d] | –P—[b] X[b] |
|---|---|---|---|---|---|---|---|
| | +,+ (TP)[a] | +,- (FP)[a] | -,- (TN)[a] | -,+ (FN)[a] | | | |
| LAMP | 357/360 (99.17%) | 3/360 (0.83%) | 239/240 (98.58%) | 1/240 (0.42%) | 0.997 | 0.988 | *0.208 $X^2$ = 8.434* |
| VIDAS UP | 351/360 (97.50%) | 9/360 (2.50%) | 233/240 (97.08%) | 7/240 (2.92%) | 0.980 | 0.963 | |
| ISO | 353/360 (98.06%) | 7/360 (1.94%) | 236/240 (98.33%) | 4/240 (1.67%) | 0.989 | 0.971 | |

[a] +,+: True positive (TP); +,-: False positive (FP); −,-: True negative (TP); −,+: False negative (FP);
[b] Likelihood ratio.
[c] Sensitivity = TP/(TP + FN).
[d] Specificity = TN/(TN + FP).

sensitivity values were found to be insignificant among the three methods ($p \geq .05$), the highest specificity and sensitivity values obtained from the LAMP method, followed by ISO and VIDAS UP. The true positive values of LAMP, VIDAS UP, and ISO methods were found as 99.17% (357/360), 97.50% (351/360), and 98.06% (353/360), and true negative values were found as 99.58% (239/240), 97.08% (233/240), and 98.33% (236/240) respectively. LAMP method was found to have higher true positive and true negative values compared to the other two methods.

In different food samples, the sensitivity and specificity values of the three methods used in this study are given in Table 2. Specificity and sensitivity values of the three methods used in this study were found as 1.00 in beef meat and minced lamb meat. Sensitivity value of the LAMP method was found as 0.984 only for soybean sprout, and 1.00 for the other food samples used in the study. Sensitivity value of the ISO method was found lower than 1.00 only for the cheese sample, however, the sensitivity values of the VIDAS UP method were found lower than 1.00 for milk and soybean sprouts. In milk samples, VIDAS UP method was significantly lower than other methods ($p < .05$), although for other food samples there were no significant differences observed ($p > .05$). Lowest sensitivity value of the three methods used in this study was found in the cheese sample of the ISO method (0.934), the lowest specificity value was obtained from the milk sample of the VIDAS UP method (0.881).

In Table 3, the specificity and sensitivity values of the three methods at different spike levels and food samples are given. It was found that the specificity values at the highest spike level (4.58 $\log_{10}$ CFU/g-ml) were 1.00. Sensitivity value of the LAMP method at the highest spike level was found lower than 1.00. Sensitivity values of the VIDAS UP method for the milk and soybean sprouts and cheese sample of the ISO method were found lower than 1.00. The sensitivity values of the LAMP and VIDAS UP methods in soybean sprouts and cheese sample of the ISO method at moderate (2.32 $\log_{10}$ CFU/g-ml) and low (0.30 $\log_{10}$ CFU/g-ml) spike levels were found lower than 1.00. Lowest sensitivity value was found in the cheese sample (0.810) in the ISO method, while the lowest specificity value was found in milk sample (0.881) in the VIDAS UP method at low spike level.

## 4. Discussion

*E. coli* O157:H7 causes hemolityic uremic syndrome and hemorragic colitis in humans and currently there is no effective treatment available for these diseases. Then, precautions and food surveillance systems against these diseases and preventing them in humans are vital (Zhang et al., 2016). Rapid, reliable, and sensitive methods are necessary for applying an effective food surveillance system. However, some factors, such as detection and isolation method, different type of sample, and so on, have major impact on the detection performance and prevalance findings of the *E. coli* O157 (Aminul Islam et al., 2006). For this purpose, in the last decades, some methods have been developed and LAMP method is one of the important of these methods (Hara-Kudo et al., 2008).

There was no significant difference between specifity and sensitivity of three methods used in this study (Table 1). Among these methods with equal specificity and sensitivity values, LAMP stands out with some features. In this method, results can be obtained in a shorter time than classical cultural methods (Zhao et al., 2010; Ngwa et al., 2013; Hara-Kudo et al., 2007). In contrast to the VIDAS UP method, it is also an important advantage that LAMP method does not require any expensive devices or instrumentations (Zhao et al., 2010). In addition, it was shown that cross-reactivity could be seen with other types of bacteria such as *Escherichia hermannii*, *Salmonella* O30 group, *Hafnia alvei*, and *Citrobacter freundii* in VIDAS UP analysis (Garci'a-Aljaro et al., 2005). On the other hand, it was found that the specificity and sensitivity values of VIDAS UP method were statistically lower than the LAMP method in some food matrices (Table 3). These results show that the LAMP method is superior to the VIDAS UP method for the detection of *E. coli* O157:H7.

In this study, the specificity and sensitivity values of the three methods in beef and minced lamb meat were found to be 1.00 (Table 2).

**Table 2**
Specificity and sensivitiy values and *P* values of LAMP, ISO, and VIDAS UP methods on *Escherichia coli* O157:H7 in food matrices (n: 60 for positive control, n: 40 for negative control).

| | LAMP | | ISO | | VIDAS UP | | |
|---|---|---|---|---|---|---|---|
| Food sample | Sensitivity[a] | Specificity[b] | Sensitivity[a] | Specificity[b] | Sensitivity[a] | Specificity[b] | -P-[c]X[b] |
| Beef meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | – |
| Minced lamb meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | – |
| Milk | 1.00 | 1.00 | 1.00 | 1.00 | 0.948 | 0.881 | *0.026 $X^b$ = 11.856* |
| Cheese | 1.00 | 0.976 | 0.934 | 0.923 | 1.00 | 1.00 | *0.611 X2 = 2.957* |
| Apple puree | 1.00 | 0.952 | 1.00 | 0.952 | 1.00 | 0.930 | *0.999 X2 = 0.440* |
| Soybean sprouts | 0.984 | 1.00 | 1.00 | 0.952 | 0.937 | 0.973 | *0.349 X2 = 6.259* |

abcd: Means in the same column with different superscripts are statistically different (p > .05)
[a] Sensitivity = TP/(TP + FN).
[b] Specificity = TN/(TN + FP).
[c] Likelihood Ratio.

**Table 3**
Specificity and Sensivitiy values of LAMP, ISO, and VIDAS UP methods detection of *Escherichia coli* O157:H7 in different spike levels in food matrices (n: 20 for positive control samples, n: 40 for negative control samples) .

| | | Spike level | | | | | |
|---|---|---|---|---|---|---|---|
| | | 4.58 $\log_{10}$ CFU/g-ml (High) | | 2.32 $\log_{10}$ CFU/g-ml (Moderate) | | 0.30 $\log_{10}$ CFU/g-ml (Low) | |
| Method | Food sample | Sensitivity[a] | Specificity[b] | Sensitivity[a] | Specificity[b] | Sensitivity[a] | Specificity[b] |
| LAMP | Beef meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Minced Lamb meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Milk | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Cheese | 1.00 | 1.00 | 1.00 | 0.976 | 1.00 | 1.00 |
| | Apple puree | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.952 |
| | Soybean sprouts | 0.952 | 1.00 | 0.952 | 1.00 | 0.952 | 1.00 |
| VIDAS UP | Beef meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Minced lamb meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Milk | 0.870 | 1.00 | 0.870 | 1.00 | 0.833 | 0.881 |
| | Cheese | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Apple puree | 1.00 | 1.00 | 1.00 | 0.930 | 1.00 | 1.00 |
| | Soybean sprouts | 0.833 | 1.00 | 0.833 | 1.00 | 0.826 | 0.973 |
| ISO | Beef meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Minced lamb meat | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Milk | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| | Cheese | 0.833 | 1.00 | 0.833 | 1.00 | 0.810 | 0.923 |
| | Apple puree | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.952 |
| | Soybean sprouts | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.952 |

[a] Sensitivity = TP/(TP + FN).
[b] Specificity = TN/(TN + FP).

Similar with our results, Hara-Kudo et al. (2008) reported that sensitivity and specificity values of the LAMP method for the detection of *E. coli* O157 in the ground beef samples were found 1.00 and 0.983, respectively. It is also stated that the LAMP method is superior or equal to classical cultural methods. In this study, the specificity value obtained from beef samples was found to be higher than the values obtained by Hara-Kudo et al. (2008). In our study, in soybean sprouts the sensitivity value at the high spike level was found as 0.952 (Table 3), in the study conducted by Hara-Kudo et al. (2008) sensitivity values was found to be 1.00 for alfalfa sprout at high spike level. In addition, in the same study, a non-inoculated ground beef and 3 alphaalfa sprout samples were positive for IMS-plating on CT-SMAC method and negative for LAMP method. In this study, in the beef and soybean sprouts there were no false negative results in the ISO method, whereas in the LAMP method only one false negative result was found in soybean sprout samples. It was though that the differences between these studies caused by different spike levels or different food matrices (ground beef and alfaalfa sprouts). Aminul Islam et al. (2006) reported in their study on 360 meat samples that there was no difference between the VIDAS UP and the IMS method for the detection of *E. coli* O157. Similarly, Vernozy-Rozand et al. (1997) were found that there were no differences found between the VIDAS UP and IMS method for the detection of *E. coli* O157 in minced beef and raw milk cheese samples. Similar with these studies results, in our study, it was found that there were no differences found for the sensitivity and specificity values among three methods in the beef, minced lamb meat and cheese samples (Table 2).

Among the three different methods used in this study, the highest sensitivity and specificity values was found in the LAMP method (Table 1). Similar to the results of this study, Hara-Kudo et al. (2007) reported that LAMP method has higher sensitivity values than other methods. In addition, Wang et al. (2012b) reported that the LAMP method has 100% specificity and sensitivity values in ground beef samples and no false negative and false positive results. In a different study Wang et al. (2012a) reported that no false-positive and false-negative results found in the LAMP method in the ground beef, beef trim, and produce samples. Similar to the results of these studies, in our current study, specificity and sensitivity values were found to be 1.00 in beef and minced lamb meat samples and false negative and false positive results were not obtained. It was reported in a different study conducted by Wang et al. (2014) that there were no false negative and false positive results in produce samples in LAMP method. However, in our study, false positive (0.83%) and false negative (0.42%) results were obtained in LAMP method. It was though that the differences between our study and Wang et al. (2014) study may be caused by using different primers (*stx*$_1$, *stx*$_2$, *eae*), different food matrices (lettuce, spinach, and sprouts), and different spike levels.

## 5. Conclusions

It can be summarized from results of this study that specificity and sensivity of the LAMP method is higher than ISO and VIDAS UP methods. In addition, this methods is less time-consuming when compared to the other methods used in this study. In conclusion, using a simple, fast, and inexpensive detection method such as LAMP in developing countries and especially in endemic regions to control outbreaks and the spreads of food borne pathogens, is crucial.

## Declaration of Competing Interest

The authors declare that there is no conflict of interests in this study.

## Acknowledgements

This study was held by the support of Pamukkale University Scientific Project Unit given the code 2018HZDP022. We would like to thank Asist. Prof. Şükrü Gökhan ELÇİ for proof reading and his supports on our research.

## References

Aminul Islam, A., Heuvelink, A.E., Talukder, K.A., de Boer, E., 2006. Immunoconcentration of Shiga toxin-producing *Escherichia coli* O157 from animal faeces and raw meats by using Dynabeads anti-*E. coli* O157 and the VIDAS UP system. Int. J. Food Microbiol. 109, 151–156. https://doi.org/10.1016/j.ijfoodmicro.2006.01.010.

Carvalho, R.N., de Oliveira, A.N., de Mesquita, A.J., Minafra e Rezende, C.S., de Mesquita, A.Q., Romero, R.A.M., 2014. PCR and ELISA (VIDAS UP ECO O157®) *Escherichia coli* O157:H7 identification in Minas Frescal cheese commercialized in Goiânia, GO. Braz. J. Microbiol. 45 (1), 7–10. https://doi.org/10.1590/s1517-83822014000100002.

Chen, L., Guo, J., Wang, Q., Kai, G., Litao Yang, L., 2011. Development of the visual loop-

mediated isothermal amplification assays for seven genetically modified maize events and their application in practical samples analysis. J. Agric. Food Chem. 59 (11), 5914–5918. https://doi.org/10.1021/jf200459s.
Conrad, C.C., Stanford, K., McAllister, T.A., Thomas, J., Reuter, T., 2014. Further development of sample preparation and detection methods for O157 and the top 6 non-O157 STEC serogroups in cattle feces. J. Microbiol. Methods 105, 22–30. https://doi.org/10.1016/j.mimet.2014.06.020.
Garcı'a-Aljaro, C., Bonjoch, X., Blanch, A.R., 2005. Combined use of an immunomagnetic separation method and immunoblotting for the enumeration and isolation of *Escherichia coli* O157 in wastewaters. J. Appl. Microbiol. 98, 589–597. https://doi.org/10.1111/j.1365-2672.2004.02497.x.
Gehring, A.G., Albin, D.M., Irwin, P.L., Reed, S.A., Tu, S., 2006. Comparison of enzyme-linked immunomagnetic chemiluminescence with U.S. food and drug administration's bacteriological analytical manual method for the detection of *Escherichia coli* O157:H7. J. Microbiol. Methods 67, 527–533. https://doi.org/10.1016/j.mimet.2006.04.025.
Hara-Kudo, Y., Nemoto, J., Ohtsuka, K., Segawa, Y., Takatori, K., Kojima, T., Ikedo, M., 2007. Sensitive and rapid detection of vero toxin-producing *Escherichia coli* using loop-mediated isothermal amplification. J. Med. Microbiol. 56, 398–406. https://doi.org/10.1099/jmm.0.46819-0.
Hara-Kudo, Y., Konishi, N., Ohtsuka, K., Hiramatsu, R., Tanaka, H., Konuma, H., Takatori, K., 2008. Detection of verotoxigenic *Escherichia coli* O157 and O26 in food by plating methods and LAMP method: a collaborative study. Int. J. Food Microbiol. 122, 156–161. https://doi.org/10.1016/j.ijfoodmicro.2007.11.078.
International Organization for Standardization-ISO 16654, 2003. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Escherichia coli* O157. Geneva, Switzerland.
Jiang, K., Zhu, Y., Liu, W., Feng, Y., He, L., Guan, W., Hu, W., Shi, D., 2012. Development of a loop-mediated isothermal amplification (LAMP) for the detection of F5 fimbriae gene in enterotoxigenic *Escherichia coli* (ETEC). Curr. Microbiol. 65, 633–638. https://doi.org/10.1007/s00284-012-0204-6.
Jung, B.Y., Jung, S.C., Kweon, C.H., 2005. Development of a rapid immunochromatographic strip for detection of *Escherichia coli* O157. J. Food Prot. 68, 2140–2143. https://doi.org/10.4315/0362-028X-68.10.2140.
Kouguchi, Y., Fujiwara, T., Teramoto, M., Kuramoto, M., 2010. Homogenous, real-time duplex loop-mediated isothermal amplification using a single fluorophore-labeled primer and an intercalator dye: its application to the simultaneous detection of Shiga toxin genes 1 and 2 in Shiga toxigenic *Escherichia coli* isolates. Mol. Cell. Probes 24, 190–195.
Kumar, S., Mondal, K.K., 2015. Visual detection of *Escherichia coli* contamination in milk and fruit juice using loop-mediated isothermal amplification. J. Food Sci. Technol. 52 (11), 7417–7424. https://doi.org/10.1016/j.mcp.2010.03.001.
Law, J.W.-F., Ab Mutalib, N.-S., Chan, K.-G., Lee, L.-H., 2015. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front. Microbiol. 5, 770. https://doi.org/10.3389/fmicb.2014.00770.
Maruyama, F., Kenzaka, T., Yamaguchi, N., Tani, K., Masao Nasu, M., 2003. Detection of bacteria carrying the stx2 gene by in situ loop-mediated ısothermal amplification. Appl. Environ. Microbiol. 69 (8), 5023–5028. https://doi.org/10.1128/AEM.69.8.5023-5028.2003.
Mori, Y., Notomi, T., 2009. Loop-mediated isothermal amplifi cation (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J. Infect. Chemother. 15, 62–69. https://doi.org/10.1007/s10156-009-0669-9.
Ngwa, G.A., Schop, R., Weir, S., León-Velarde, C.G., Odumeru, J.A., 2013. Detection and enumeration of *E. coli* O157:H7 in water samples by culture and molecular methods. J. Microbiol. Methods 92, 164–172. https://doi.org/10.1016/j.mimet.2012.11.018.
Niessen, Ludwig, Luo, Jie, Denschlag, Carla, Vogel, Rudi F., Food Microbiology, December 2013. The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants. Food Microbiology 36 (2), 191–206.
Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28 (12), E63. https://doi.org/10.1093/nar/28.12.e63. https://www.sciencedirect.com/science/article/pii/S0740002013000865.
O'Brien, S.B., Duffy, G., Daly, D., Sheridan, J.J., Blair, I.S., McDowell, D.A., 2005. Detection and recovery rates achieved using direct plate and enrichment/immunomagnetic separation methods for *Escherichia coli* O157:H7 in minced beef and on bovine hide. Lett. Appl. Microbiol. 41, 88–93. https://doi.org/10.1111/j.1472-765X.2005.01667.x.
Oporto, B., Esteban, J.I., Aduriz, G., Juste, R.A., Hurtado, A., 2008. *Escherichia coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* in healthy cattle, sheep and swine herds in northern Spain. Zoonoses Public Health 55, 73–81. https://doi.org/10.1111/j.1863-2378.2007.01080.x.
Quilliam, R.S., Williams, A.P., Avery, L.M., Malham, S.K., Jones, D.L., 2011. Unearthing human pathogens at the agricultural–environment interface: a review of current methods for the detection of *Escherichia coli* O157 in freshwater ecosystems. Agric. Ecosyst. Environ. 140, 354–360. https://doi.org/10.1016/j.agee.2011.01.019.
Sasitharan, D., Selvam, M.M., Manohar Paul, W., M, W., 2013. Loop-mediated isothermal amplification for diagnosis of *Escherichia coli* O157:H7 and viewpoints on its progression into realistic point of care. Int. J. Chem. Environ. Biol. Sci. 1 (2), 244–247.
Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.-A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States—major pathogens. Emerg. Infect. Dis. 17 (1), 7–15. https://doi.org/10.3201/eid1701.p11101.
Sidari, R., Caridi, A., 2011. Methods for detecting enterohaemorrhagic *Escherichia Coli* in food. Food Rev. Int. 27, 134–153. https://doi.org/10.1080/87559129.2010.535232.
SPSS, IBM Corp. Released, 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: USA.
Vernozy-Rozand, C., Mazuy, C., Ray-Gueniot, S., Boutrand-Loei', S., Meyrand, A., Richard, Y., 1997. Detection of *Escherichia coli* 0157 in French food samples using an immunomagnetic separation method and the VIDAS UP™ *E. coli* O157. Lett. Appl. Microbiol. 25, 442–446. https://doi.org/10.1111/j.1472-765X.1997.tb00014.x.
Wang, F., Jiang, L., Yang, Q., Prinyawiwatkul, W., Ge, B., 2012a. Rapid and specific detection of *Escherichia coli* serogroups O26, O45, O103, O111, O121, O145, and O157 in ground beef, beef trim, and produce by loop-mediated isothermal amplification. Appl. Environ. Microbiol. 78, 2727–2736. https://doi.org/10.1128/AEM.07975-11.
Wang, F., Jiang, L., Ge, B., 2012b. Loop-mediated isothermal amplification assays for detecting Shiga toxin-producing *Escherichia coli* in ground beef and human stools. J. Clin. Microbiol. 50 (1), 91–97. https://doi.org/10.1128/JCM.05612-11.
Wang, F., Yang, Q., Qu, Y., Meng, J., Ge, B., 2014. Evaluation of a loop-mediated isothermal amplification suite for the rapid, reliable, and robust detection of Shiga toxin-producing *Escherichia coli* in produce. Appl. Environ. Microbiol. 80 (8), 2516–2525. https://doi.org/10.1128/AEM.04203-13.
Wang, T., Kima, S., An, J.H., 2017. A novel CMOS image sensor system for quantitative loop-mediated isothermal amplification assays to detect food-borne pathogens. J. Microbiol. Methods 133, 1–7. https://doi.org/10.1016/j.mimet.2016.12.002.
Yano, A., Ishimaru, R., Hujikata, R., 2007. Rapid and sensitive detection of heat-labile I and heat-stable I enterotoxin genes of enterotoxigenic *Escherichia coli* by loop-mediated isothermal amplification. J. Microbiol. Methods 68, 414–420. https://doi.org/10.1016/j.mimet.2006.09.024.
Zhang, X., Li, Y., Li, B., Mao, Y., Wu, X., Zou, X., Gao, P., Yan, H., Yang, D., Ling, Y., Chen, H., 2016. Three supplementary methods for analyzing cytotoxicity of *Escherichia coli* O157:H7. J. Microbiol. Methods 120, 34–40. https://doi.org/10.1016/j.mimet.2015.11.011.
Zhao, X., Li, Y., Wang, L., You, L., Xu, Z., Li, L., He, X., Liu, Y., Wang, J., Yang, L., 2010. Development and application of a loop-mediated isothermal amplification method on rapid detection *Escherichia coli* O157 strains from food samples. Mol. Biol. Rep. 37, 2183–2188.
Zhao, X., Wang, J., Forghani, F., Park, J.-H., Park, M.-S., Seo, K.-H., Oh, D.-H., 2013. Rapid detection of viable *Escherichia coli* O157 by coupling propidium monoazide with loop-mediated isothermal amplification. J. Microbiol. Biotechnol. 23 (12), 1708–1716.