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Recent trends in the utilization of LAMP for the diagnosis of viruses, bacteria, and allergens in food

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1 Introduction

Early identification of foodborne pathogens is a necessary aspect to regulate the illness and mortality rate of humans as they are caused mainly by foodborne pathogenic bacteria (Rohde et al., 2017; Wang et al., 2017). Currently, more than 200 diseases are occurring due to the consumption of unsafe, contaminated, and undercooked food (Viswanath et al., 2018). Food substances lead to allergic reactions in both children and adults. These include soybean, eggs, peanuts, fish, wheat, crustaceans, and cereals, which mainly consist of gluten (Boyce et al., 2010; Chafen et al., 2010). Furthermore, the outbreaks of foodborne pathogens run across developed countries like the U.S., Asia, and Europe, where industrialization is high (Lake and Barker, 2018). The significant diseases caused by them and the socio-economic loss, the diagnosis of foodborne pathogenic bacteria and viruses against spoilage of food have become a challenge of public health (Wang et al., 2017). The existing conventional methods available for the detection of foodborne pathogens are expensive, time-consuming, and laborintensive (Law et al., 2015). To address these issues, biosensors are acting as promising alternatives for the detection of foodborne pathogens. These offer good selectivity and more sensitivity within a short period of time in contrast to other conventional methods (Zhao et al., 2014). Different biomolecules such as monoclonal antibodies (mAbs), lectin, glycan, and single-stranded DNA (ssDNA) act as bioreceptors. These will distinguish the target biomarker, which in turn is a characteristic feature for specific bacterial pathogens (Vidic et al., 2017; Evtugyn et al., 2017). Up to date, several electroanalytical and optical biosensors have been developed. But they are limited to the bench-top and cannot develop as potential portable point-of-care (POC) devices. To address these issues, LAMP has revolutionized molecular biology as a highly compatible and flexible POC platform. LAMP has several advantages such as (i)

highly sensitive and fast responsive, (ii) cost-effective and ease in obtaining information, (iii) high throughput, and (iv) practicable in POC settings (Zhang et al., 2014). Most of the foodborne diseases occur by bacteria including Escherichia coli, Campylobacter, Salmonella enterica, Vibrio cholerae, and Listeria, and viruses such as Norovirus (NoV) and hepatitis A virus. Consumption of undercooked meat, uncleaned vegetables, poultry products, and other animal products like eggs and unpasteurized milk are major sources of foodborne diseases. More importantly, seafood such as fish, crab, prawn, shrimp, and squid are acting as the major reservoirs for viral pathogens. The common symptoms of these foodborne diseases include abdominal pain, nausea, vomiting, diarrhea, headache, etc. (Bhardwaj et al., 2017; Torok et al., 2018). In 2013, FoodNet reported more than 19,000 cases of foodborne infections, in which 80 deaths and 4200 hospitalizations occurred in the U.S. Foodborne diseases involved in the annual economic burden approximately \$78 billion dollars and devastated the socioeconomic status of the country by the uncontrolled foodborne outbreaks. Hence, accurate diagnosis of foodborne diseases is a significant phenomenon in the current research. For this purpose, a robust and powerful analytical technique, i.e., LAMP has emerged for nucleic acid amplification, which operates at a steady temperature (60°C–65°C), within 30–60 min using DNA primers. From the development of the LAMP method by Notomi et al. in 2000 until now, several LAMP-based methodologies have played major key roles in the detection of foodborne pathogens. LAMP has emerged as a valuable and reliable method for both qualitative and quantitative diagnosis of foodborne diseases caused by various foodborne pathogens such as bacteria, viruses, and other food allergens (Guevara et al., 2016; Tomita et al., 2008; Focke et al., 2013). A list of foodborne bacteria, viruses, and other food allergens and related databases are illustrated in Fig. 1.

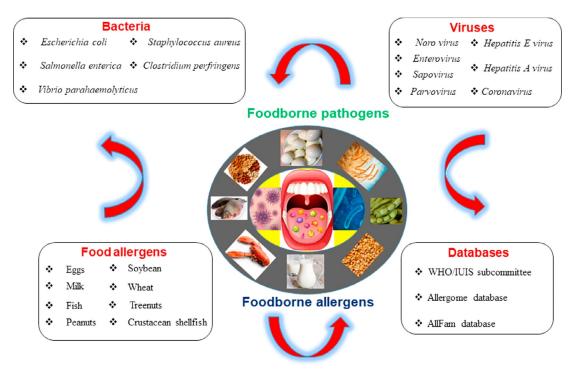


FIG. 1 Overview of pathogens and allergens responsible for foodborne infections and existing databases for foodborne allergens.

2 Lamp in the detection of food bacteria

Concerning food safety, a potential detection strategy is necessary for the evaluation and early determination of foodborne pathogens. In favor of these issues, polymerase chain reaction (PCR) is one of the hopeful analytical techniques for identifying target genes from the foodborne pathogens. But, as PCR is expensive and time-consuming, there must be alternatives to develop, i.e., LAMP. Interestingly, LAMP offers some advantages compared with the PCR such as (i) rapid operation, (ii) high sensitivity, (iii) maximum specificity, and (iv) cost-effectiveness for the detection of foodborne pathogens. In addition, LAMP has emerged as a hotspot analytical technique for real-time analysis of foodborne pathogens due to their beneficial factors such as (i) fluorescence, (ii) turbidity, and (iii) electrochemical sensing capabilities. Despite its high specificity and rapidity compared with PCR, LAMP is not up to the task for POC diagnosis. To resolve these issues, many researchers are focusing on the integration of complementary-metal oxide-semiconductor (CMOS) technology with LAMP. By this, real-time reaction monitoring has been successfully achieved. Wang et al. developed a CMOS-based LAMP system, which enables real-time monitoring of photon changes accordingly to the amplification process. For this propose, Hydroxynaphthol blue (HNB) was utilized as a metal indicator, which can change color from violet to blue color. Later, the red LED was employed as the light source, which accounts for a decline in photon count. In this report, a well-known pathogen E. coli O157 was selected as a target to evaluate the limit of detection (LOD) and quantitative analysis via CMOS technology (Wang et al., 2017). A promising multitarget LAMP technology was developed by employing a droplet microfluidic method for the detection of foodborne pathogens. In this technique, complicated microfluidic structures are not utilized, but only DNA sample mixtures and LAMP buffers tend to emulsiy and, after that, pico-injected. Furthermore, amplification results were obtained by counting the number of droplets. In this regard, this technique advantageous when compared to the chamber-based microfluidic LAMP, as it requires real-time fluorescence detection instruments. Interestingly, the founded LOD also is very low, i.e., 21 copies/µL. This is almost 500 times lower LOD than obtained with bulk-phase LAMP. In addition, this technique is able to detect bacterial DNA in wastewater samples, which proves its maximum detection speed and high sensitivity. Moreover, this work is able to detect multipathogenic DNAs with a very low LOD value (Yuan et al., 2018a, 2018b). In another study, Li et al. developed the viable but nonculturable (VBNC) state-based LAMP methodology by using DNA-intercalating dyes such as ethidium monoazide (EMA) and propidium monoazide (PMA). As developed, PMA/EMA-LAMP-based technology was successfully employed for the quantification of VBNC state of bacteria. It demonstrated a magnificent impact on the understanding of the epidemics, where people severely suffer from a broad variety of foodborne infections. It has shown (i) high sensitivity, (ii) maximum specificity, and (iii) rapidity over the conventional PCR-based methods (Li et al., 2017a, b). Multiplex efficiency and high-throughput screening for the detection of multiple pathogenic bacteria employing a single device in foodborne infections are always highly recommendable. To fulfill these benefits, the centrifugal LAMP microdevice was developed for the identification of foodborne bacteria such as E. coli O157: H7, Salmonella enterica, and Vibrio parahaemolyticus. Herein, the authors designed the centrifugal microdevice and the RPM control sophisticatedly. Thereby it allows the simultaneous performance of 24 LAMP reactions in a high-throughput manner. These reactions can be easily distinguished by the naked eye through examining the Eriochrome Black T (EBT)-mediated colorimetric color change from purple to sky blue. They employed the RGB-based ratiometric image processing strategy facilitates to obtain higher LOD values compared with other electrochemical and fluorescence-based detection methods. Furthermore, this method can be employed as the POC diagnostic platform for the detection and identification of foodborne bacteria in the future (Seo et al., 2017). Recently, paper-based microfluidic chips have gained much interest in the detection and screening of multiplex foodborne pathogens. Pang et al. designed a novel self-priming polydimethoxysiloxane (PDMS)/paper hybrid microfluidic chip, which shortly termed as SPH chip, integrated with mixed-dye-based LAMP technology for the detection of multiplex foodborne pathogens. This chip has the following advantages such as (i) cost-effective chip-based multiplex pathogens detection, (ii) simple operation through SPH chip, and (iii) accurate mixed-dye-based LAMP methodology. The LOD values for Staphylococcus aureus and Vibrio parahemolyticus were found to be 21.5 and $20.9 \text{ copies } \mu L^{-1}$, respectively. Furthermore, practicality was assessed by using food contaminants, and 1000 CFUmL⁻¹ bacteria can be detected with high specificity (Pang et al., 2018). Fig. 2 illustrates the schematic representation of current LAMP-based technologies for the detection of foodborne pathogens. In another study, the lab-on-a-disc device was fabricated by employing LAMP reagents and constructed into a single microfluidic compact disc (CD) with the automation process. This device has several beneficial factors when compared with the existing conventional methods. These include (i) time-saving process, i.e., within 70min, (ii) versatility accounts for the usage of microfluidic operation and hot air gun, (iii) no need of external pumps for the manipulation of LAMP assay, and (iv) eliminated the use of laboratory instruments such as PCR cabinet, vortex, PCR tubes, pipette, mixing equipment, etc., by automated microfluidic mixing of overall LAMP reagents. The currently developed portable device was employed for the detection of salmonella (spiked on the tomatoes) and achieved the LOD value of 5×10^{-3} ng/µL DNA concentration. This microfluidic lab-on-a-disc

integrated with LAMP is highly convenient in the POC of foodborne pathogen diagnostics (Sayad et al., 2016). Coming to the most specific foodborne pathogen, Clostridium perfringens is one of the significant widespread foodborne bacteria in both developed and developing countries and mainly accounts for food poisoning. Especially, in India, its prevalence is high; around 30%-60% have been observed in birds and animals. By minding the concerns, authors developed a LAMP methodology with 100% specificity, and LOD was found was to be 0.34 pg. Furthermore, high sensitivity was achieved with a LOD of 1.2×10^2 CFU/ g in 6h of enrichment and 1.2×10^5 CFU/g without enrichment in the artificial spiking recovery investigations. Enhanced detection of C. perfringens, i.e., 12 CFU/g within 12h proved that the proposed LAMP assay is faster than existing traditional methods that take >2 days. This method is highly recommendable for the detection of C. perfringens strains in food, clinical, and environmental samples even in POC diagnostic settings (Priya et al., 2018).

3 Foodborne virus detection via lamp

Viruses are the versatile microbial pathogens leading to the epidemic of severe acute respiratory syndrome (SARS), avian flu, and swine flu pandemic in 2003, 2005, and 2009, respectively. They can create the worst situations in human health within a short period of time (Ksiazek et al., 2003; Ferguson et al., 2005; Smith et al., 2009; Dawood et al., 2009). In today's world, viruses are the vulnerable foodborne pathogens causing vast damage to human health. In this context, NoV, it immensely causes acute gastroenteritis, which accounts for 1 in 5 cases among developed countries. The Centers for Disease Control and Prevention (CDC) conducted a comprehensive assessment of gastroenteritis outbreaks in the U.S. between the years 2009 and 2012. It clearly suggested that approximately 48% of foodborne disease outbreaks were reported, which are caused by the NoV (Bosch et al., 2018). Generally, molecular-based assays are frequently employed for the detection of viral nucleic acids. However, these are limited in the detection of viruses due to the following aspects: (i) lack of abundant highly infectious viruses, which require critical sampling and assessing of huge volumes of food; (ii) requirement of extraction and concentration of virus samples before detection; and (iii) the need for the extraction of viruses, which are free from interfering substances with virus detections assays or methods. On the other hand, direct detection and proper identification of viruses in food products is also a difficult task owing to the wide variety and complexity of foods and the other heterogeneous distribution of viruses in the contaminated food substances. Therefore, molecular detection methods do not clearly distinguish the presence of infectious viruses, other than the fragments of the viral genome. Most of the viruses

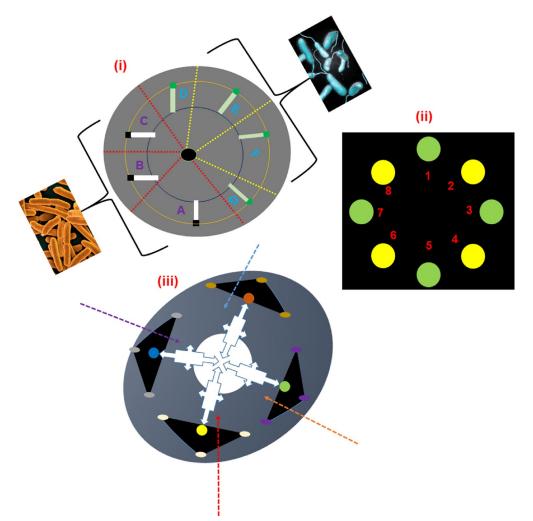


FIG. 2 Schematic representation of centrifugal LAMP microdevice in which (i) A, B, and C (purple color—dark gray color in print version) show the positive reactions, and D, E, F, and G (light blue color—gray color in print version) demonstrate the negative reactions for primers; (ii) the mixed-dye-loaded reaction assay in LAMP; and (iii) LAMP-integrated microfluidic chip.

associated with foodborne infections include NoV and Hepatitis A virus (HAV). However, other viruses such as Hepatitis E virus (HEV), Astrovirus, Human Rotavirus (HRV), Aichi virus, Enterovirus, Sapovirus, Parvovirus, Coronaviruses, and Adenovirus can be transmitted via food substances. Depending on the symptoms of infection, viruses can be grouped into three groups, i.e., (i) those that cause gastroenteritis (NoV, Astroviruses, Aichi virus, HRV, Sapoviruses, and Adenoviruses), (ii) enterically transmitted hepatitis (caused by HEV and HAV, which transmit to the liver and manifest disease), and (iii) those that mainly replicate in the human intestine but cause illness after the migration to organs, i.e., the central nervous system (Enterovirus) (WHO, 2008). Most of the 10 families of viruses are causing food-borne infections. The diseases range from simple diarrheal infections to severe inflammations in the brain, i.e., encephalitis. The global burden of foodborne infections is highly progressing due to human viral transmissions via very poor hygienic practices (Scallan et al., 2011). Among the viruses, Hepatitis viruses are one of the heavily responsible for foodborne infections, which include hepatitis A and E viruses. The cost of the burden for foodborne hepatitis A is estimated to be greater than \$36,000 U.S. per one individual in the United States. Hepatitis A can be transmitted via the fecal-oral route and ingestion of highly contaminated water and foods including fruits, shellfish, and uncooked or unprocessed vegetables. More importantly, outbreaks of foodborne and waterborne infections related to hepatitis A virus are responsible for 2%-7% of the total diseases (Sánchez, 2013). Hepatitis E virus is also one of the major types of hepatitis virus causing foodborne infections. Four genotypes of the virus and genotypes 3 and 4 are zoonotic viruses that infect humans, pigs, and other related animals. Acute hepatitis E virus outbreaks happened several times in the world. Mortality rates are observed in 25% in pregnant women in the third trimester. This virus is transmitted by means of the fecal-oral route via the consumption of contaminated food and water. This infection is mainly attributed to the consumption of raw or undercooked meat obtained from boar and deer, liver sausages, and commercial livers. Recently, reverse transcription LAMP (RT-LAMP) methods were developed that integrate with gold nanoparticles for the detection of hepatitis E viruses. This one-step RT-LAMP combined with colorimetric labeling strategy successfully can be employed to detect hepatitis E viruses compared with the existing traditional detection methods. This method is highly recommendable due to its simplicity, rapidity, and high sensitivity along with a detection limit of almost 10¹ RNA copies (Chen et al., 2014; Bartolo et al., 2015; Neethirajan et al., 2017). NoVs are one of the significant viral types that cause foodborne infections very frequently. For this, RT-LAMP assay was developed, which demonstrated outstanding specificity and high selectivity owing to the six primers, where two loop primers identified eight regions in the target sequence of the virus. In most of the cases, this assay has reduced the assay amplification time within 60 or 90min (Fukuda et al., 2006).

4 Detection of food allergens through lamp-based technologies

Food allergens are a posing serious threat to individuals by causing immune reactions and other complications. The major reason for this issue is the development of sensitization in individuals who are consuming allergenic foods. To mitigate these problems, the government of the United States introduced one-act, i.e., the Food Allergen Labeling and Consumer Protection Act in 2004 (FALCPA 2004, Public Law 108-282, Title II). According to this, all food manufacturers must label their products, whether they contain eight significant allergenic foods including eggs, fish, milk, tree nuts, peanuts, soybeans or wheat, and crustacean shellfishes. These "big eight" are highly crucial for almost 90% of all food allergies. In North America, the calculated peanut, fish, and milk allergy rates for adults are 0.6%, 0.4%, and 0.3%, respectively. In case of young children, allergens include cow's milk (2.5%), peanuts (1%), tree nuts (0.5%), soy (0.4%), wheat (0.4%), fish (0.1%), shellfish (0.1%), sesame (0.1%), and egg (1.5%). Whereas, allergic reactions to fruits and vegetables are also not uncommon (5%) (Sicherer and Sampson, 2006, 2010). Accordingly, several databases have been developed to address the causative and preventive measures required for these allergies. Among these are (i) the WHO/IUIS Subcommittee on Allergen Nomenclature (http://www. allergen.org/), (ii) the Allergome database (http://www. allergome.org/), and (iii) AllFam database (http://www. meduniwien.ac.at/allergens/allfam/). However, the detection of food allergens is not an easy task due to the following reasons: (i) presence of food allergens in trace amounts in some foods and (ii) interference of the matrix. Currently, numerous analytical methods are available such as biosensors based on electrochemical, fluorescence, and other impedance principles. All these methods have their own demerits including (i) lack of POC diagnostics, (ii) lack of early diagnosis of food allergens, (iii) expensiveness, (iv) improper screening of allergens, (v) well-experienced personnel, and (vi) lack of sophisticated equipment (Alves et al., 2015). Hence, to solve these issues, ready-to-use technology like LAMP is reliable and dependable, which provides opportunistic progress in the advancement of detection strategy for the food allergens. Sheu et al. developed a LAMP assay for the detection of allergic peanuts by employing primer sets. The two sets of the LAMP primers targeted on the internal transcribed sequence 1 (ITS1) of nuclear ribosomal DNA and the arah1 gene sequence in the peanuts. The authors confirm that the identification of peanut through freshly designed primers for the ITS 1 sequence is highly sensitive compared with the primers for the sequence of Arah1 gene via LAMP assay. This LAMP assay demonstrated high specificity in the identification of the peanut and, interestingly, no cross-reaction to other species of nut such as almonds, cashew, hazelnut, and walnut was observed (Sheu et al., 2018). In another study, the colorimetric microfluidic-LAMP chip was developed successfully or the highly selective detection of three allergens including peanut, soybean, and sesame with outstanding sensitivity and specificity within 60 min. Furthermore, this method can be employed for testing the presence or absence of food allergens that are purchased in the market. Interestingly, its accuracy is well enough compared with the conventional Taq Man real-time PCR method (Yuan et al., 2018b).

5 Limitations for lamp-based methods in the detection of foodborne pathogens

Recently, LAMP-based technologies play an impressive role in the detection of foodborne pathogens including bacteria, viruses, and other food allergens. In addition, LAMP allows the label-free and real-time detection of lethal foodborne pathogens in foodborne infections. However, LAMP has faced some limitations in both the POC and clinical diagnostic settings. These limitations include (i) maintenance of controlled environment for designing the LAMP-based microfluidic devices, (ii) requirement of well-experienced personal for the fabrication of integrated LAMP-based chips, (iii) automation of LAMP in prior and after the completion of the reaction, (iv) choice of primer sets, and (v) accurate diagnosis of nearly related species among the more complex foodborne pathogens. Additionally, it may be difficult to maintain suitable aspects such as primer selectivity and other operational conditions. In this manner, advanced integrated LAMP-based systems are highly appropriate to detect foodborne pathogens (Li et al., 2017a, b; Ma et al., 2019).

6 Conclusion and future prospectives

Global burden increases due to the raising of major foodborne disease outbreaks, which are caused by bacteria, viruses, and other related allergens worldwide. However, some of the molecular diagnostic approaches such as PCR is showing great impact; having some disadvantages are not satisfactory. These include moderate specificity, expensiveness, and need for sophisticated instruments. In addressing these issues, the advanced and potential integrating systems such as LAMP-based microdevices, microchips, and other CD-LAMP-based systems are developed today. These systems allow high specificity, rapidity, multiplex foodborne pathogen detection, and maximum sensitivity. In addition, these LAMP-based systems provide opportunistic pathogen detection in POC diagnostics. These draw a clear distinguishing mark in the diagnosis of pathogens from the clinical side to the bedside. Finally, LAMP technology can be effectively employed for the easy and systematic diagnosis of various foodborne pathogens such as bacteria, viruses, and related allergens.

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Conflict of interest

The authors declare no conflicts of interest.

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