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## Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases

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**Abstract** Loop-mediated isothermal amplification (LAMP) is an established nucleic acid amplification method offering rapid, accurate, and cost-effective diagnosis of infectious diseases. This technology has been developed into commercially available detection kits for a variety of pathogens including bacteria and viruses. The current focus on LAMP methodology is as a diagnostic system to be employed in resource-limited laboratories in developing countries, where many fatal tropical diseases are endemic. The combination of LAMP and novel microfluidic technologies such as Lab-on-a-chip may facilitate the realization of genetic point-of-care testing systems to be used by both developed and developing countries in the near future. This review will describe the historical, current, and future developments of such technologies.

**Key words** Loop-mediated isothermal amplification (LAMP) · Nucleic acid amplification test · Rapid diagnosis tool · Point of care testing (POCT) device

### Introduction

Despite progress in medical science, including the development of effective chemotherapies, infectious diseases continue to affect millions of lives around the world, especially in developing countries.<sup>1</sup> Diagnosis is important not only for the prescribing of effective drugs for appropriate patients in adequate doses but also for preventing the evolution of resistant microorganisms, which occurs by treating noninfected people who show similar symptoms.<sup>2</sup> Therefore, the development of rapid, accurate, and sensitive diagnostic methods for the identification of pathogens is fundamental for treating and controlling, or even eradicating, infectious disease.

Classical pathogen detection and identification is based on culture methodology and microscopy. The culture method is still a core technology in clinical laboratories because the method can also provide important information about the viability of the pathogen and its susceptibility to antibiotics.<sup>3</sup> However, the slow multiplication and difficulty of selective cultivation of many important pathogens often limits the culture-based diagnosis.<sup>4</sup> The direct observation of microbes or parasites by microscopy is frequently employed as a rapid and simple diagnostic method. The robustness and cost-efficiency of microscopic tests make them acceptable to use even in resource-limited laboratories in developing countries. However, the poor sensitivity of smear tests often causes fatal delays in treatment and the methodology is heavily dependent upon the skill of the microscopist.<sup>5</sup> Viral infection has also been diagnosed by selective cultivation, followed by electron-microscopy observation. A major drawback is the delay of treatment in patients at infirmaries in both developed and developing countries.<sup>6,7</sup> Accordingly, the establishment of more rapid, sensitive, and accurate diagnostic methods has long been desired.

In the past few decades, several molecular methods have been developed to overcome the shortcomings of the classical diagnostic methods mentioned above,<sup>8</sup> especially, the *in vitro* amplification of a pathogen-specific nucleic acid sequence. Such approaches may allow rapid diagnosis with a degree of sensitivity and specificity comparable to or even better than that of classical culture methods. Many amplification methods, including polymerase chain reaction (PCR), ligase chain reaction (LCR), nucleic acid sequence-based amplification (NASBA), and strand displacement amplification (SDA) have already been commercialized as kits and introduced into routine diagnostics as nucleic acid amplification tests (NATs).<sup>9</sup> NATs can offer additional advantages over conventional methods; for instance, by the facilitation of standardization and automation coupled with an ability to type species and detect drug resistance.<sup>10</sup>

Since loop-mediated isothermal amplification (LAMP) was originally reported by Notomi et al. in 2000,<sup>11</sup> LAMP has attracted a lot of attention as a potentially rapid,

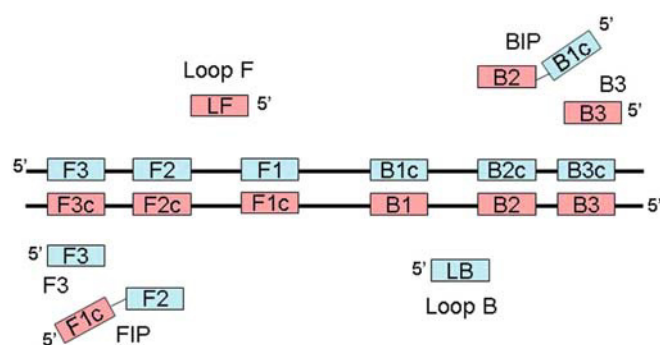
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accurate, and cost-effective novel nucleic acid amplification method. As a result, more than 180 reports evaluating the LAMP method have now been published in many publications, including this journal.<sup>12</sup> The LAMP method has now been developed as commercial kits and some of them have been adopted as the officially recommended methods for the routine identification and surveillance of pathogens in Japan. As such, the LAMP method must be recognized as an established nucleic acid amplification method for NAT. In this article, we review the history of LAMP development for the rapid diagnosis of infectious diseases. The current directions of development as well as the future prospects of LAMP are also discussed.

## History of the development of the LAMP method

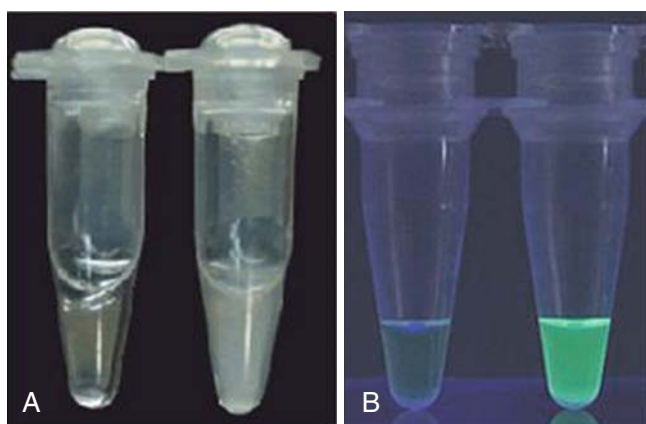
### Development of basic technology

LAMP is a one-step amplification reaction that amplifies a target DNA sequence with high sensitivity and specificity under isothermal conditions. The mechanism of the LAMP reaction can be explained in three steps, an initial step, a cycling amplification step, and an elongation step. An animation on the website is useful for better understanding of the principle.<sup>13</sup> LAMP employs a DNA polymerase with strand-displacement activity, along with two inner primers (FIP, BIP) and outer primers (F3, B3) which recognize six separate regions within a target DNA (Fig. 1). The LAMP assay has high specificity, because the amplification reaction occurs only when all six regions within a target DNA are correctly recognized by the primers. The addition of reverse transcriptase makes it possible to amplify DNA from RNA sequences (RT-LAMP). While PCR has a wide range of applicability as the fundamental tool for molecular biology methods such as molecular cloning, the LAMP method has many characteristics that make it suitable for the rapid and simple detection of nucleic acid sequences in samples. Optimization of the LAMP method as a rapid diagnostic tool has been done as follows.



**Fig. 1.** Schematic representation of loop-mediated isothermal amplification (LAMP) primers. The inner primers FIP (BIP) are composed of F2 (B2) and F1c (B1c). The outer primers are designed at the region of F3 and B3. The loop primers are designed between F1c (B1c) and F2c (B2c)

- So-called Loop-primers (LF, LB), which are additional primers designed to anneal at the loop structure in LAMP amplicons, can accelerate and enhance the sensitivity of the LAMP reaction (Fig. 1).<sup>14</sup> The loop primers are now commonly used in the practical applications of LAMP.
- Real-time turbidity measurement by a cost-effective photometer with incubation function has enabled the kinetic analysis of the LAMP reaction without the need for any detection reagents such as a fluorescence intercalator. This is because the LAMP reaction produces insoluble magnesium pyrophosphate in the course of the amplification reaction. It has been reported that the real-time turbidity analysis of LAMP makes it possible to quantify the initial amount of template DNA in the samples.<sup>15</sup> Furthermore, a LAMP positive reaction can be easily detected by visual endpoint judgment of turbidity (Fig. 2A).<sup>16</sup> The development of such a simple detection method as well as an inexpensive real-time turbidimeter has contributed to the acceptance of the LAMP amplification method.
- Recently, an effective endpoint detection method has been developed by Tomita et al.<sup>17</sup> using calcein, whose fluorescence is quenched by the binding of manganese ions (Fig. 2B). Because the manganese ions are bound by pyrophosphate ions produced in the course of the amplification reaction, calcein produces bright fluorescence in a positive reaction. The simplicity and clarity of this method should make it applicable even for resource-limited laboratories in rural areas of developing countries.
- It has been shown that LAMP exhibits less sensitivity to inhibitory substances present in biological samples than PCR.<sup>18</sup> This robustness of LAMP against inhibitors can contribute to saving the time and cost required for sample processing steps.



**Fig. 2A,B.** Visual endpoint detection method of LAMP reaction. **A** Detection of LAMP reaction by turbidity. *Left*, without template (negative); *right*, with template DNA (positive). Template: plasmid DNA inserted with polymerase chain reaction (PCR) product for mRNA of prostate-specific antigen. **B** Detection of LAMP reaction by fluorescence using calcein. *Left*, without template (negative); *right*, with template (positive). Template: severe acute respiratory syndrome corona virus (SARS-CoV) RNA

Sometimes, the difficulty of designing LAMP primers keeps researchers away from trying to use LAMP in their research work. The free software for designing LAMP primers (Primer Explore; Fujitsu, Tokyo, Japan) can provide candidate LAMP primers for a target sequence.<sup>19</sup> The latest version of the software can take into account mutations in the target sequence for designing either mutation-sensitive or -insensitive primers.

#### Application of LAMP for detecting various pathogens

In the initial phase of development, LAMP has been applied to many kinds of pathogens causing food-borne diseases.<sup>20</sup> LAMP kits for detecting *Salmonella*, *Legionella*, *Listeria*, verotoxin-producing *Escherichia coli*, and *Campylobacter* have been commercialized.

*Salmonella* is a major food-borne pathogen that causes human gastrointestinal and other diseases.<sup>21</sup> *Salmonella* is transmitted by contaminated food products such as eggs, milk, poultry, and vegetables. In particular, the serotype Enteritidis has been recognized as a cause of infection via contaminated eggs.<sup>22</sup> The conventional culture-based confirmation method for *Salmonella* is time-consuming (approximately 5 days) and requires labor-intensive procedures for nonselective pre-enrichment, selective enrichment, and biochemical and serological tests. Although PCR-based methods have been applied for the sensitive and rapid detection of *Salmonella* in enrichment cultures,<sup>23</sup> they require the electrophoretic analysis of amplicons or the use of an expensive thermal cycler with a fluorescence detector.<sup>24</sup> Hara-Kudo et al.<sup>25</sup> have developed a LAMP assay for detecting the *Salmonella enterica* invasion protein gene in enrichment culture from liquid egg samples. They have compared the sensitivity of LAMP and PCR assays and reported that their LAMP assay had higher sensitivity (−2.2 cfu/test) than that of PCR. Ohtsuka et al.<sup>26</sup> have applied the above-mentioned LAMP assay to liquid egg samples collected from food processing plants. They have also reported that the LAMP assay was not inhibited by the constituents of liquid egg because of the tolerance of Bst polymerase to inhibitors. Of note, Okamura et al.<sup>27</sup> have developed a LAMP assay for amplifying the fragments of the O9 *Salmonella*-specific insertion elements.

*Escherichia coli* is another important pathogen causing food-borne disease.<sup>28</sup> Hara-Kudo et al.<sup>29</sup> have evaluated a commercially available LAMP assay kit for verotoxin-producing *E. coli* (VT-screening kits; Eiken Chemical, Tokyo, Japan). They have demonstrated that the VT gene from O-157 or O-26 in enrichment culture can be detected by the LAMP assay kit with similar or superior sensitivity to those of some plating methods using agar media. Yano et al.<sup>30</sup> have developed LAMP primer sets for the rapid and sensitive diagnosis of enterotoxigenic *E. coli* infection. Furthermore, Hill et al.<sup>31</sup> have developed a LAMP assay for detecting common strains of *E. coli* from human urine samples with high sensitivity and specificity. The LAMP method has also been applied for the diagnosis of *Campylobacter coli*.<sup>32</sup>

Noroviruses (NoVs) are also important food-borne pathogens causing human gastroenteritis diseases worldwide.<sup>33</sup> Oysters are often associated with food-borne outbreaks of NoVs.<sup>34</sup> In addition, NoVs can cause outbreaks by person-person transmission in institutional settings such as schools and hospitals.<sup>35</sup> Because NoVs cannot be cultured, the detection and identification of NoVs is carried out by electron microscopy, which is less sensitive and time-consuming.<sup>36</sup> Thus, many molecular diagnostic methods have been developed using reverse transcription (RT) PCR,<sup>37</sup> nucleic acid sequence-based amplification (NASBA),<sup>38</sup> and transcription-reverse transcription concerted (TRC) amplification.<sup>39</sup> In routine laboratory work, the diagnosis of NoVs is often performed by RT-PCR or real-time RT-PCR using sequence-specific detection. Although such PCR assays have high sensitivity and can distinguish genotypes (GI and GII), they are time-consuming and require expensive instrumentation. For improving such practices, LAMP assay kits for detecting NoVs and distinguishing the GI and GII genotypes have been commercially developed. Iturriza-Gomara et al.<sup>40</sup> have evaluated the kits using fecal samples collected from patients and reported that the LAMP kits for GI and GII performed well. They concluded that the lower sensitivity of the kit for GI strains than for GII strains would have little impact on the laboratory diagnosis of NoVs because the GII strain causes the majority of outbreaks. Some research groups have reported their original LAMP assay for detecting NoVs from fecal specimens.<sup>41–43</sup> Furthermore, Fukuda et al.<sup>44</sup> have reported a novel approach of two-step isothermal amplification for detecting NoVs in oysters. Because the numbers of copies of the NoV genome in oysters are low,<sup>45</sup> this group employed NASBA as a pre-amplification of template RNA to enhance the sensitivity of RT-LAMP.

The clinical application of LAMP has been shown with a severe acute respiratory syndrome corona virus (SARS-CoV) detection kit, which has been evaluated by Poon et al.<sup>46</sup> They have concluded that the overall performance of the LAMP kit for nasopharyngeal aspirate samples is comparable to that of optimized real-time RT-PCR assays. Although the detection rate of the LAMP kit for samples from the first 3 days after disease onset was found to be slightly worse than that of the RT-PCR assay, the simplicity of the LAMP assay may have potential for clinical diagnosis in the field or bedside situation. The application of LAMP to SARS-CoV detection has also been reported by Hong et al.<sup>47</sup> They have succeeded in designing LAMP primers with 100-fold-greater sensitivity compared to the conventional RT-PCR method.

Influenza is a major public health problem.<sup>48</sup> Human influenza is caused by infection with the influenza A or B virus. Of 15 hemagglutinin subtypes and 9 neuraminidase subtypes of influenza A, only 3 hemagglutinin subtypes (H1-H3) and 2 neuraminidase subtypes (N1, N2) can infect humans. Influenza can cause fatal disease in the very young and elderly people unless appropriately treated.<sup>49</sup> Because chemotherapy based on a neuraminidase inhibitor, oseltamivir, has been found to be effective when this agent is administered within 24 h after onset of fever,<sup>50,51</sup> rapid diag-

nostic methods are required not only for achieving optimal benefit but also for preventing overtreatment with the medicine. Ito *et al.*<sup>52</sup> have reported that their LAMP primer sets for influenza subtype 1 (HA1), subtype 3 (HA3), and influenza B virus have a higher sensitivity and specificity than those of commercially available rapid diagnostic tests (RDTs).

In addition to human influenza, highly pathogenic avian influenza (subtype H5N1) has been considered as an important problem for both human health and economics.<sup>53</sup> For preparedness against outbreaks of a novel avian influenza virus which acquires human-human transmission ability, the development of laboratory diagnostic tools with high sensitivity and simplicity has been desired. The LAMP assay for the detection of H5 avian influenza virus from throat swab specimens collected from wild birds was first reported by Imai *et al.*<sup>54</sup> They have shown that their primer sets designed on the H5 hemagglutinin gene can detect H5N1 influenza virus with 100-fold higher sensitivity than the RT-PCR, and the primers do not amplify RNA from the human influenza virus (H1N1 and H3N2). They have also applied their assay to detect H5N1 virus from throat swabs collected from patients clinically suspected of being infected with the virus.<sup>55</sup> Furthermore, Jayawardena *et al.*<sup>56</sup> have reported that the detection limit of their LAMP assay was identical to that of an optimized RT-PCR assay recommended by the World Health Organization (WHO).

LAMP assays have been developed for the diagnosis of other important pathogens, including measles virus,<sup>57</sup> human papilloma virus,<sup>58</sup> mumps virus,<sup>59,60</sup> *Cryptosporidium* oocysts,<sup>61</sup> *Legionella*,<sup>62</sup> and *Vibrio cholerae*.<sup>63</sup>

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## Current direction of development

The greatest need for a simple and field-friendly diagnostic tool is in developing countries, where populations are exposed to many dangerous infectious diseases.<sup>64</sup> The current direction of the development of LAMP is toward a simple diagnostic tool which can be routinely employed in the poorly resourced laboratories in rural areas of developing countries.

Tuberculosis (TB) is one of the leading causes of infectious disease in developing countries.<sup>65</sup> Although the morbidity of TB has decreased in many developed countries, the incidence has been rising in developing countries. One of the reasons for failure to control TB in developing countries is the lack of affordable simple diagnostic methods that have better sensitivity than the sputum smear test commonly used in the resource-limited rural laboratories.<sup>66</sup> Although NAT systems for TB have become common in developed countries, there is no NAT system that is simple enough and affordable enough to be acceptable in the rural laboratories of developing countries.<sup>67</sup> Iwamoto *et al.*<sup>68</sup> have reported a LAMP assay that can detect *Mycobacterium tuberculosis* as well as *M. avium* and *M. intracellulare*. This assay has almost the same sensitivity as that of the PCR-based Amplicor system (Roche Diagnostics GmbH: Basel,

Switzerland) which tests sputum samples decontaminated by N-acetylcysteine-NaOH treatment.<sup>68</sup> Boehme *et al.*<sup>69</sup> have reported the evaluation of a LAMP assay designed for use in peripheral microscopic laboratories in developing countries. In their study, the LAMP method with a filter-based sample pretreatment method for raw sputum was demonstrated to have enough simplicity for use in resource-limited settings and to have almost the same clinical performance as that of commercialized NAT for TB.

Malaria is another serious threat to the health of children and pregnant women in the developing world.<sup>70</sup> Annually, 300–500 million clinical episodes of malaria occur, resulting in about 2 million deaths.<sup>71</sup> Rapid diagnosis is important for malaria patients because the symptoms of malaria overlap with those of many other infections.<sup>72</sup> Of the four malaria species that infect humans, *Plasmodium falciparum* requires prompt detection because it causes the most severe and fatal disease.<sup>72</sup> Antimalarial drugs are often prescribed presumptively to patients suspected of having malaria, without any diagnosis.<sup>71</sup> This practice of overtreatment accelerates the evolution of *Plasmodium* isolates with drug resistance. For these reasons, rapid and accurate diagnostic methods are becoming essential for malaria control and prevention. At present, the Giemsa-stained thick- and thin-blood film microscopy test is considered to be the gold standard. However, the method is labor-intensive and requires well-trained experts.<sup>73</sup> Although some RDTs have been commercially available, none of them has higher sensitivity and discrimination for the four species than conventional microscopy.<sup>74</sup> PCR is highly sensitive and can differentiate malaria species. However, the cost, time, and laboratory infrastructure requirements make this unrealistic for peripheral laboratories in developing countries.<sup>5</sup> Poon *et al.*<sup>85</sup> have reported a LAMP assay that can detect *P. falciparum* directly from simple heat-treated blood with a sensitivity of ~6 parasites/ $\mu$ l blood, which is almost ten times higher than that of the conventional blood smear test (~50 parasites/ $\mu$ l blood) carried out by experienced microscopists. Poon *et al.*<sup>85</sup> have concluded that the LAMP assay with simple heat treatment of blood samples is an option for the molecular diagnosis of *P. falciparum* even in basic healthcare settings in developing countries. On the other hand, Han *et al.*<sup>75</sup> have developed LAMP primer sets that allow the discrimination of all four species, as well as allowing diagnosis at genus level. The performance of their assays has been similar to those of nested PCR in terms of sensitivity and specificity. They have also demonstrated that a simple water bath can be used as an incubator for the LAMP reaction. Their LAMP assay might provide an alternative to conventional microscopy for the routine diagnosis of malaria at remote clinics in developing countries.<sup>76</sup> These reports have shown that the LAMP method with a simple blood treatment method is a promising technology for malaria control in developing countries.

In addition to LAMP assays for TB and malaria, a LAMP assay has also been developed for human immunodeficiency virus (HIV), which is the other major agent causing fatal disease in developing countries.<sup>77</sup> Furthermore, LAMP has also been applied for the diagnosis of human African

trypanosomiasis (HAT) or sleeping sickness, which is known as a neglected tropical disease.<sup>78</sup> HAT is caused by infection with *Trypanosoma brucei rhodiense* or *T. b. gambiense*, which are transmitted by tsetse flies. Because HAT is fatal if left untreated, rapid and accurate diagnosis is essential. Both the diagnosis and the staging of the disease must be accurate because the drugs used against *T. b. rhodiense* and *T. b. gambiense* have unacceptable toxicity.<sup>79</sup> Although some methodologies have been proposed for the field diagnosis of HAT, parasite detection remains insufficiently sensitive for decreasing the prevalence of the disease.<sup>80</sup> The application of LAMP to HAT detection was reported first by Kuboki et al. in 2003.<sup>81</sup> Later, the same group (Thekisoe et al.<sup>82</sup>) reported that their re-designed primer sets could distinguish even subspecies. Recently, Njiru et al.<sup>83</sup> have reported a LAMP assay that can detect *T. b. rhodiense* with high sensitivity of 10 parasites/ml blood. They have also succeeded in detecting the subgenus *Trypanozoon* with an extremely high sensitivity of 0.001 parasites/ml blood, using the repetitive insertion mobile element (RIME) sequence as the target.<sup>84</sup> Furthermore, it has been confirmed that simple heat treatment of blood<sup>75</sup> can be effective in HAT detection. The evaluation of LAMP for other tropical diseases such as dengue virus,<sup>86</sup> Ebola virus,<sup>87</sup> Chikungunya virus,<sup>88</sup> and West Nile virus<sup>89</sup> has also been reported.

There is no currently available NAT system with enough simplicity, robustness, and cost-effectiveness for common use at the poorly resourced laboratories in developing countries. As mentioned above, LAMP and its peripheral technologies, including quick and simple sample processing methods, are now in development for the establishment of the simplest NAT system for diagnostics in developing countries. Such a diagnostic method could be of great benefit to those living in developing countries where many diseases are endemic.

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## Future prospects

The development of the above-mentioned simple NAT system could have potential as a genetic point-of-care testing device or bedside diagnostic tool to eradicate or control infectious diseases in developed countries. The worldwide panic caused by the pandemic of SARS in 2002 has clearly shown that communicable disease is still a problem even in developed countries. It is likely that the same kind of panic will be produced by pandemics of other emerging diseases or diseases such as H5 influenza. The worldwide airlines have been providing tourists with easy access to areas that are endemic for many diseases,<sup>90</sup> and global warming may help the spread of vector-borne diseases to nonendemic areas.<sup>91</sup> In order to combat such epidemics, it is necessary to establish diagnostic tools that can be used anywhere, by anyone, and at anytime to screen patients.

Recently, new technologies such as the Lab-on-a-chip have been developed and applied to various fields of analytical science.<sup>92</sup> The concept of the Lab-on-a-chip can be

explained as a miniaturized analytical laboratory constructed on a small chip, which allows all the analytical steps from sample pretreatment to detection to be conducted in a rapid, effective, and automatic format. NAT is one of the promising applications of the Lab-on-a-chip technology because NAT consists of successive labor-intensive procedures, sample processing (extraction of target nucleic acid), amplification of the target sequence, and detection of amplicons. Although the concept of Lab-on-a-chip has already been applied to PCR,<sup>93</sup> the above-mentioned characteristics of LAMP may have the potential to facilitate the development of Lab-on-a-chip-based NAT devices.

A novel integrated isothermal device for real-time turbidity measurement of the LAMP reaction has been proposed by Lee et al.<sup>94</sup> Their device consists of a disposable polymer microreactor and a base unit with a thin-film heater for temperature regulation, a light-emitting diode as a light source, and a phototransistor as an optical detector. Using the device, they have quantitatively detected hepatitis B virus (HBV) from clinical serum samples by kinetic analysis of LAMP reactions. The viral load is important information for treating patients with chronic infectious disease.<sup>95</sup> Their device could have the potential to be a simple and low-cost medical device or point-of-care testing device for monitoring the progress of chronic viral infectious disease.

Hataoka et al.<sup>96</sup> have achieved the integration of LAMP and subsequent ultrafast electrophoresis analysis of amplicons on a commercially available polymer microchip. The isothermal condition of LAMP enables the use of a cost-effective polymer chip instead of glass or silicon, which is often used for PCR-based chips, to endure the high temperatures of thermal cycling.<sup>93,97</sup> The resolution of the microchip-based electrophoresis is high enough to distinguish between amplicons with four different base pairs. Therefore, two different amplicons from each target can be detected independently in the same analysis. Accordingly, this system could be a useful detection method for multiplex LAMP amplification.<sup>98</sup> The development of novel sequence-specific detection methods for LAMP could facilitate the establishment of Lab-on-a-chip-based NAT systems. Nakamura et al.<sup>99</sup> have developed an electrochemical DNA chip for the sequence-specific detection of LAMP amplicons, where the oxidation of an electrochemical active intercalator is monitored on a gold electrode with immobilized oligo-DNA probes. Their method is time-saving and cost-effective compared with a conventional fluorescence-based DNA chip because it does not require fluorochrome labeling and expensive fluorescence analysis equipment. Other technologies for sequence-specific detection have been reported by Mori et al.<sup>100</sup> and Tani et al.<sup>101</sup>

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## Concluding remarks

In this article, the historical, current, and future directions of LAMP technology for the diagnosis of infectious diseases have been reviewed. The motivation for the development of LAMP technology is to provide a rapid, cost-effective,

and accurate diagnosis for patients in any situation. Such a technology will surely contribute to quality of life now and in future for patients in both developed countries and locations where diseases are endemic.

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