



# An innovative and user-friendly smartphone-assisted molecular diagnostic approach for rapid detection of canine vector-borne diseases

Archana Upadhyay<sup>1,2</sup> · Raza Muhammad Waleed<sup>3</sup> · Jinhua Wang<sup>1,4</sup> · Jianguo Zhao<sup>1,2</sup> · Qingfeng Guan<sup>1,2</sup> · Chenghong Liao<sup>1,2</sup> · Qian Han<sup>1,2</sup> 

Received: 24 June 2020 / Accepted: 1 February 2021 / Published online: 2 March 2021  
© The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature 2021

## Abstract

Present-day diagnostic tools and technologies for canine diseases and other vector-borne parasitic diseases hardly meet the requirements of an efficient and rapid diagnostic tool, which can be suitable for use at the point-of-care in resource-limited settings. Loop-mediated isothermal amplification (LAMP) technique has been always a method of choice in the development and validation of quick, precise, and sensitive diagnostic assays for pathogen detection and to reorganize point-of-care (POC) molecular diagnostics. In this study, we have demonstrated an efficient detection system for parasitic vector-borne pathogens like *Ehrlichia canis* and *Hepatozoon canis* by linking the LAMP assay to a smartphone via a simple, inexpensive, and a portable “LAMP box.” All the components of the LAMP box were connected to each other wirelessly. This LAMP box was made up of an isothermal heating pad mounted below an aluminum base which served as a platform for the reaction tubes and LAMP assay. The entire setup could be connected to a smartphone via an inbuilt Wi-Fi that allowed the user to establish the connection to control the LAMP box. A 5 V USB power source was used as a power supply. The sensitivity of the LAMP assay was estimated to be up to  $10^{-6}$  dilution limit using the amplified, purified, and quantified specific DNA templates. It can also serve as an efficient diagnostic platform for many other veterinary infectious or parasitic diseases of zoonotic origin majorly towards field-based diagnostics.

**Keywords** Canine · *Ehrlichia canis* · *Hepatozoon canis* · LAMP box · Parasitic vector-borne diseases · Point-of-care

## Introduction

Pathogenic organisms are the agents of infectious diseases, and their transmission can be threatening in all respects and

can result in economic losses. Promising diagnostic methods are therefore needed for the proper diagnosis and management of such diseases. In such cases, point-of-care (POC) tests can be a boon especially in resource-limited or field settings. Nucleic acid (NA) detection using point-of-care (POC) is a rapidly evolving field which has great application in clinical and commercial setups (Auroux et al. 2002; Yager et al. 2008; Lui et al. 2009; Arora et al. 2010; Kovarik et al. 2013; Gauglitz 2014; Sackmann et al. 2014). NA-based POC diagnostic platforms require NA isolation and purification before proceeding for amplification (Easley et al. 2006), and these are very important and influential parameters. Polymerase chain reaction (PCR) is the most routinely used method for NA amplification which undergoes multiple thermo-cycling steps for complete amplification of NA. On the contrary, isothermal amplification of NA drastically cuts down the thermo-cycling stages, which is more economical without compromising with the quality of DNA (Asiello and Baeumner 2011; Chang et al. 2013; Yan et al. 2014). Many interesting and efficient technological advances in POC

---

Section Editor: Leonhard Schnittger

---

✉ Chenghong Liao  
liaoqh@hainanu.edu.cn

✉ Qian Han  
qianhan@hainanu.edu.cn

- <sup>1</sup> Laboratory of Tropical Veterinary Medicine and Vector Biology, School of Life and Pharmaceutical Sciences, Hainan University, Haikou 570228, Hainan, China
- <sup>2</sup> One Health Institute, Hainan University, Haikou 570228, Hainan, China
- <sup>3</sup> College of Computer and Cyberspace Security, Computer Science and Technology, Hainan University, Haikou 570228, Hainan, China
- <sup>4</sup> College of Animal Science and Technology, Hainan University, Haikou 570228, Hainan, China

testing can play a vital role in patient care and public health care by providing user-friendly, handy, smarter, rapid, economical, and pervasive diagnostic setups on the go (Jani and Peter 2013; Vashist et al. 2014; Vashist et al. 2015). Subsequently, many such newly developing nucleic acid amplification test (NAAT) devices are shifting from the classical laboratory-based platforms to mobile, comprehensive, lucid, and economical POC testing platforms (Yang and Rothman 2004; Chin et al. 2012; Jiang et al. 2014; Priye and Ugaz 2016). Loop-mediated isothermal amplification (LAMP) has popped up to be a very popular isothermal NAAT for NA detection due to its less complex thermal steps and higher sensitivity (Notomi et al. 2000; Notomi et al. 2015; Song et al. 2016; Tian et al. 2016). Moreover, isothermal NAATs do not necessarily require thermal cyclers, thereby eliminating the need of complex hardware and software's for such kind of tests. For end-point result analysis, LAMP assay generally relies on turbidity monitoring and using indicators like hydroxynaphthol blue (HNB) (Lau et al. 2015), SYBR green dye as a post-reaction analysis (Iwamoto et al. 2003), or agarose gel electrophoresis (Notomi et al. 2000). Apart from LAMP assay, there have been many other assays like the rolling circle amplification (RCA) (Monsur Ali et al. 2014), strand displacement amplification (SDA) (Shi et al. 2014), signal-mediated amplification of RNA technology (SMART) (Hall et al. 2002), nucleic acid sequence-based amplification (NASBA) (Compton 1991), single primer-triggered isothermal amplification (Ma et al. 2015), helicase-dependent amplification (HDA) (Vincent et al. 2004), and cross priming amplification (CPA) (Xu et al. 2012; Craw and Balachandran 2012; Chang et al. 2013) which are used to achieve isothermal amplification of NA. Still, LAMP has been faster, more stable, and sensitive for NA detection (Notomi et al. 2000; Notomi et al. 2015). Interestingly, LAMP-based methods have shown to produce >50-fold more amplicon than PCR-based techniques (Seyrig et al. 2011). Apart from this, LAMP assay provides a flexibility to amplify medium- to long-range template strands of nucleic acids (>130 bp and <300 bp), which makes it highly favorable for amplification of NA of several infectious pathogens (Notomi et al. 2000). LAMP assay amplification can give good results even in the presence of certain inhibitors (Abdul-Ghani et al. 2012) and some food ingredients (Kaneko et al. 2007; Wang et al. 2008; Kiddle et al. 2012). The specificity of the LAMP assay is due to four to six different specific primers and a Bst polymerase enzyme used for amplification (Notomi et al. 2000; Parida et al. 2008; Craw and Balachandran 2012; Tanner and Evans Jr. 2014). Many significant advances have been developed for detecting the LAMP amplicons including electrochemical detection mechanism (Zhang et al. 2014), pH sensing modality, and many more (Ahmed et al. 2008; Toumazou et al. 2013; Zhang et al. 2014).

*Hepatozoon canis* causes canine hepatozoonosis which is one of the most commonly occurring infections found in the canine populations globally (Otranto and Dantas-Torres 2010; Singh et al. 2017a). The causative parasite involved in it has a relatively complex and unique life cycle, where the infection takes place on ingesting the ixodid tick which already contains the mature oocysts of the parasite (Nava et al. 2015). The brown dog tick, *Rhipicephalus sanguineus (sensu lato)*, has been reported to be the sole vector responsible for *H. canis* infection (Gavazza et al. 2003; Nava et al. 2015). Conventional parasitological techniques such as microscopy have been used for the diagnosis of canine hepatozoonosis (Baneth and Shkap 2003). According to some previous studies, several sensitive tests such as indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) have been proven to be sensitive especially in diagnosing chronic infections (Shkap et al. 1994; Mylonakis et al. 2005). Molecular diagnostic assays such as PCR and real-time PCR have been employed for the identification and diagnosis of *H. canis* infections (Otranto et al. 2011; Abd Rani et al. 2011; Aktas et al. 2015; Singh et al. 2017a). PCR being a gold standard for diagnosis, these molecular tests win over other tests due to their high sensitivities and specificities for the detection of such infections from the host as well as the vector (Latrofa et al. 2014; Singh et al. 2017b). However, PCR assays are not easily accessible in the field, due to their higher costs and complex mechanism (Mandal et al. 2015). LAMP assays have been substantially used for the precise and timely diagnosis of various infectious agents or pathogens (Notomi et al. 2000; Parida et al. 2008; Notomi et al. 2015; Mandal et al. 2015). Furthermore, it is easier to evaluate the end-point results either by dye-based methods or by visualizing the presence of turbidity in positive reactions (Mori et al. 2001; Karanis and Ongerth 2009). In the field of veterinary diagnosis, LAMP assays have been developed and effectively used for the detection of *Babesia gibsoni* (Ikadai et al. 2004; Mandal et al. 2015), *Trypanosoma evansi* (Thekisoe et al. 2005), *Theileria equi* (Alhassan et al. 2007), *Babesia caballi* (Alhassan et al. 2007), *Cryptosporidium parvum* (Karanis et al. 2007), *Babesia orientalis* (He et al. 2009), *Toxoplasma gondii* (Krsteva et al. 2009), *Theileria parva* (Thekisoe et al. 2010), *Theileria sergenti* (Wang et al. 2010), and *Tritrichomonas foetus* (Oyhenart et al. 2013).

Canine monocytic ehrlichiosis (CME), significantly marked by the presence of clinical and hematological signs, is caused by a tick-borne bacterium called *Ehrlichia canis* (Harrus and Waner 2011). CME has been known to have similar clinical signs and hematological alterations like other tick-borne diseases which can be quite challenging for veterinarians for its diagnosis and treatment (Harrus and Waner 2011). It occurs as a multi-systemic disease, ranging from an acute to subclinical and, in some cases, leading to a chronic phase (Harrus and Waner 2011). The pathogen is widespread globally and is predominantly found in tropical and subtropical regions. Moreover, co-infection of dogs with *E. canis* and other tick-transmitted pathogens has also been

reported in such regions, where the vectors are prevalent (Mekuzas et al. 2009; Santos et al. 2009; Al Izzi et al. 2013; Eiras et al. 2013; De Tommasi et al. 2013). Several molecular diagnostic tests such as PCR, nested PCR, and real-time PCR assays have been previously used to detect *E. canis* in dogs (McBride et al. 1996; Stich et al. 2002; Doyle et al. 2005; Kledmanee et al. 2009; Nakaghi et al. 2010; Peleg et al. 2010; Cardozo et al. 2011). The 16S rRNA nested PCR assay developed for the diagnosis and detection of *E. canis* has been frequently used; however, a nested PCR assay targeting the p30 gene has been recently reported to be more sensitive (Stich et al. 2002). Despite of being highly specific and sensitive nested PCR tests, they are often judged due to their time-consuming process and cross-contamination issues, which may lead to false-positive results (Cardozo et al. 2011). On the other hand, LAMP assays are sensitive and specific and have an advantage of producing and visualizing the results in relatively lesser period of time (Notomi et al. 2000).

The gold standard nucleic acid amplification tests (NAAT), multiplexed quantitative reverse-transcription polymerase chain reaction (qRT-PCR), and many such tests require sophisticated, expensive, power-consuming, and bulky thermal cyclers (for different thermal conditions) with fluorescence detection systems (for performing real-time fluorescence detection). These techniques come with conditions like high turnaround time and complexity; hence, these factors make them less favorable for point-of-care testing and their application in fields. Recently, many evolving NAAT devices are transiting from the traditional benchtop-based detection systems towards more complete, mobile, and inexpensive field-based systems (Yang et al., 2004; Chin et al. 2012; Jiang et al. 2014; Priye and Ugaz 2016).

In the present study, we report the development of a smartphone-based rapid, accurate, and networked diagnostic LAMP box system for canine infectious diseases. This LAMP box comprises an isothermal heating pad which serves as a platform for placing the reaction tubes, heat sensor to check the temperatures, a UV, and white LED lights for visualizing the results, and then this entire setup can be connected to the smartphone via an inbuilt Wi-Fi which allows the user to establish a connection, and subsequently it leads to the simple graphical user interface (GUI) which can control the steps of reaction of the LAMP box. A 5 V USB power source was used to supply power for the LAMP assay in the box. This developed platform can be used for many such infectious diseases particularly towards field-based diagnostics.

## Materials and methods

### Sample preparation and PCR assay

Previously collected and identified positive blood samples for *E. canis* and *H. canis* from dogs of Hainan province, which

were confirmed by DNA sequencing, were processed for fresh DNA isolation (unpublished data). Total DNA was isolated using 200  $\mu$ l of EDTA-anticoagulated blood samples by using TIANamp blood DNA kit (TIANGEN, China) and Sangon Biotech Ezup blood DNA kit according to the manufacturer's protocol. DNA concentrations and purities were determined by measuring the absorbance using a NanoDrop Spectrophotometer (Thermo Scientific, USA). The extracted DNA samples were eluted in nuclease-free water and processed and/or stored at  $-20$  °C for downstream applications. The DNA samples which were isolated were subjected to amplification by PCR using previously developed PCR assays (Inokuma et al. 2002; Pinhanelli et al. 2015). The primers used and their thermal cycling conditions are summarized in Table 1.

End-point PCR assays on the basis of the 18S rRNA gene of *H. canis* and p30 gene of *E. canis* were performed, which consisted of 1  $\mu$ l of DNA solution, 12.5  $\mu$ l of 2x Buffer for TransGen Biotech PCR Super Mix, 5  $\mu$ l of 2 mM dNTPs, 3  $\mu$ l of 25 mM MgSO<sub>4</sub>, 0.5  $\mu$ l of forward primer, 0.5  $\mu$ l of reverse primer (50 pmol/ $\mu$ l each) (Table 2), 1  $\mu$ l of 2 x *EcoTaq* PCR Super Mix (1 U/ $\mu$ l), and double distilled water to make up a final volume of 25  $\mu$ l. The reaction mixtures were cycled in an Eppendorf gradient thermal cycler (Eppendorf). PCR products were examined on 2% agarose gel stained with 0.4  $\mu$ g/ml ethidium bromide using a Quick-Load 5kb DNA Ladder marker (TAKARA BIO, Inc. China), visualized under the Gel Doc XR<sup>+</sup> Imaging system (BIO-RAD Laboratories, Inc.).

### LAMP assay

The LAMP assay was carried out by using previously used primers for *H. canis* and *E. canis* as mentioned in Table 2. The LAMP reaction was performed in 25  $\mu$ l reaction volumes as described (Notomi et al. 2000), with fewer modifications. Each LAMP reaction of 25  $\mu$ l contained 9  $\mu$ l nuclease-free water, 2.5  $\mu$ l 10x Isothermal Amplification Buffer Pack (contains 20mM Tris-HCl, 10mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 50mM KCl, 2mM MgSO<sub>4</sub>, 0.1% Tween1 20) (New England Biolabs), 3.5  $\mu$ l 10 mM each dNTPs (New England Biolabs), 1  $\mu$ l 100 mM MgSO<sub>4</sub> (New England Biolabs), 3.5  $\mu$ l 5M Betaine (Affymetrix), 40 pmol each of FIP and BIP primers, 50 pmol each of the F3 and B3 primers, 1  $\mu$ l Fluorescent Detection Reagent (FD), 1.0  $\mu$ l Bst 2.0 WarmStart DNA Polymerase (8 units/ $\mu$ l) (New England Biolabs), and 1  $\mu$ l of sample DNA template. Contamination issues were resolved to its maximum by addition of mineral oil on the top part of each reaction tube. The amplification reaction was performed at 64 °C for 45 min and terminated at a high temperature of 92 °C for 2 min. Nuclease-free water was used as a negative control template. After incubation, the tubes containing the amplified DNA target were checked visually by two persons to check the color changes observed, purple color represented a

**Table 1** Primer sets with respective product sizes and thermal cycling conditions for DNA amplification of the pathogens in dogs

| Pathogen/tick species   | Gene     | Primers   | PCR conditions   | Reference                |
|-------------------------|----------|---|--|--------------------------|
| <i>Hepatozoon canis</i> | 18S rRNA | HEP-F: ATACATGAGCAAAATCTCAAC<br>HEP-R: CTTATTATTCCATGCTGCAG                 | 95 °C, 5 min; 35 cycles [95 °C 60 s, 58 °C 60 s, 72 °C 60 s]; 72 °C, 5 min | Inokuma et al. (2002)    |
| <i>Ehrlichia canis</i>  | P30      | P30-1SPF: ATGGGTGGCCCAAGAATAGA<br>ACTTG<br>P30-1SPR: CATCCTGCTATGGTTCCTAGTG | 95 °C, 5 min; 40 cycles [95 °C 45 s, 60 °C 30 s, 72 °C 30 s]; 72 °C, 5 min | Pinhanelli et al. (2015) |

positive test, whereas no color change referred to as negative control. To confirm the reaction, 15 µl of each LAMP product was examined on 2% agarose gel stained with 0.4 µg/ml ethidium bromide using a Quick-Load 5kb DNA Ladder marker (TAKARA BIO, Inc. China), visualized under the Gel Doc XR<sup>+</sup> Imaging system (BIO-RAD Laboratories, Inc.).

### Sensitivity, specificity, and diagnostic performance of LAMP assay

The isolated DNA samples were processed and were subjected to the 18S rRNA and P30 PCR assays (Table 1) for the amplification of *H. canis* and *E. canis*, followed by the

confirmation of respective amplifications by observing specific bands by agarose gel electrophoresis. The amplified PCR products were purified using the DNA gel purification kit (Sangon prep Kit) as per the manufacturer's protocol, and DNA was eluted using 30 µl of elution buffer. The concentrations and purities of the eluted samples were determined by measuring the absorbance using the NanoDrop spectrophotometer (Thermo Scientific, USA). Tenfold serial dilutions of the eluted and quantified DNA were done by using the eluted and quantified DNA (1 µl) which were used to detect the sensitivity and detection limit of the assay. The amplicons from PCR were further confirmed by 2.0% agarose gel electrophoresis containing ethidium bromide. To determine the

**Table 2** Nucleotide sequences of LAMP primers used for the detection of *H. canis* (18S rRNA gene) and *E. canis* (p30 gene)

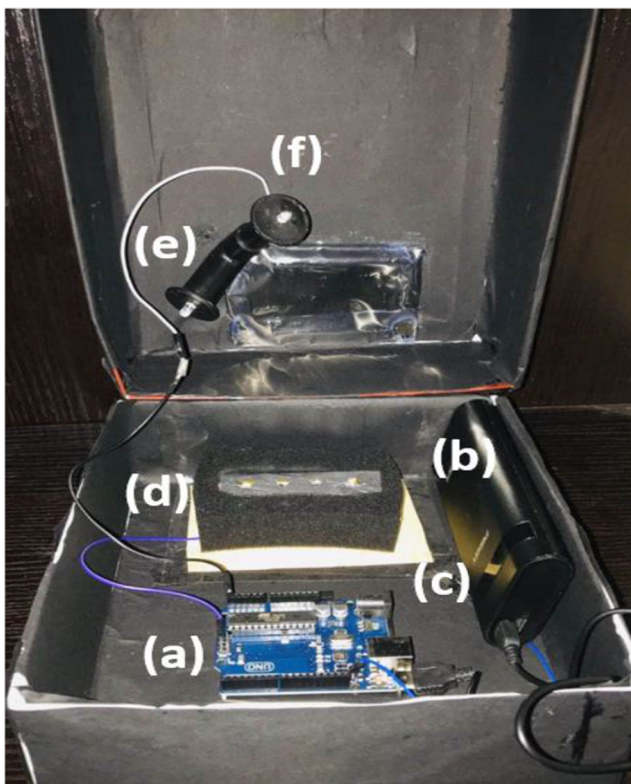
| Primers | Type                 | Genes    | Pathogen species        | Sequences (5'→3')  | References             |
|---------|----------------------|----------|-------------------------|--|------------------------|
| F3-p30  | Forward outer primer | P30      | <i>Ehrlichia canis</i>  | GGCCCAAGAATAGAACTGA  | Pinhanelli et al. 2015 |
| B3-p30  | Reverse outer primer | P30      | <i>Ehrlichia canis</i>  | CCTCAATTATTATGTCATAGCATG                                       | Pinhanelli et al. 2015 |
| Fip-p30 | Forward inner primer | P30      | <i>Ehrlichia canis</i>  | TGTGTGCGCCGTTCTTATAA<br>TTgaattcAGTTCTGTACGAGA<br>CATTCG       | Pinhanelli et al. 2015 |
| Bip-p30 | Reverse inner primer | P30      | <i>Ehrlichia canis</i>  | CATCATAGTTCAGCAACAAA<br>CATGTgaattcAATGATAAGTCAAT<br>TAACCCTTC | Pinhanelli et al. 2015 |
| F3      | Forward outer primer | 18S rRNA | <i>Hepatozoon canis</i> | GCAAAGTGAAAACAGGCG   | Singh et al. 2019      |
| B3      | Reverse outer primer | 18S rRNA | <i>Hepatozoon canis</i> | AGAATTGGGTAATTTGCGC  | Singh et al. 2019      |
| FIP     | Forward inner primer | 18S rRNA | <i>Hepatozoon canis</i> | GCCACGGTAAGCCAATACCA<br>TAAATCATTCAAGTTTCTGACCT                | Singh et al. 2019      |
| BIP     | Reverse inner primer | 18S rRNA | <i>Hepatozoon canis</i> | GTGACGGTTAACGGGGGATT<br>GTGGTAGCCGTTTCTCAG                     | Singh et al. 2019      |



specificity of the LAMP assay, a positive DNA sample of *B. canis vogeli* was also used as a DNA template for the LAMP assay to validate its specificity and cross reactivity. In order to assess the usefulness of the optimized smartphone-enabled LAMP assay in a BOX as a smart and a rapid diagnostic tool, representative samples from the previously collected, identified (positive and negative), and extracted genomic DNA from the dogs (unpublished data) were tested using this platform. The diagnostic performance of the smartphone-assisted LAMP box was evaluated using a total number of 226 DNA samples of dogs (unpublished data).

### Smartphone-assisted portable LAMP box

A 5V isothermal heating pad (COM-11288; 5VDC), UV LED light (Lite-On Inc.; Part# LTPL-C034UVH385), and white LED light (Panasonic Electronic components; Part LNJ03004BDD1) were maneuvered via an Arduino esp8266 microcontroller (ESP8266 ESP-01 Wi-Fi Arduino) which could wirelessly connect to a smartphone. Here, we used the iPhone 7 model (R-62000612). A hard cardboard box was used to assemble all the components. The inner and the outer dimensions of the box were approximately 32.5\*23\*17.5 cm and 34\*25\*18.2 cm, respectively (Fig. 1). The entire box was covered by a black film to facilitate smooth and uninterrupted



**Fig. 1** Raw image of the portable LAMP box displaying the components of the box in an open form. a Arduino esp8266, b power source, c heat sensor, d isothermal heating pad, e UV LED light, and f white LED light

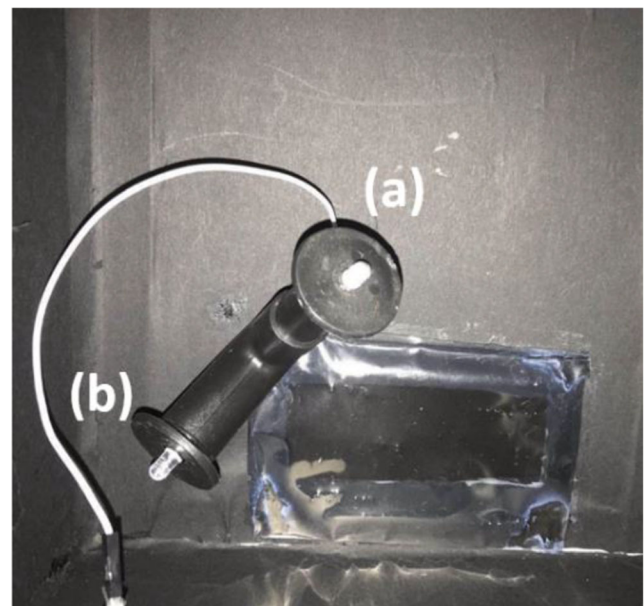
visualization of results under UV light (Fig. 2). The highly detailed description about the LAMP box has been mentioned in the Supplementary Information section (Supplementary Text 1: Smartphone-assisted portable LAMP box).

### Statistical analysis

The sensitivity of the LAMP assay was calculated at 95% confidence intervals (CI) for using the SPSS V.17.0 program.

### Results

The optimal results and appropriate reaction conditions, which were represented by a characteristic ladder-like pattern generally observed for LAMP products, were accomplished successfully at 64°C for a time period of 45 min. A series of serial dilutions of the template DNA from the amplified LAMP products of *E. canis* p30 gene and *H. canis* 18S rRNA gene, when subjected to agarose gel electrophoresis, demonstrated similar ladder-like patterns for the same. The amplified PCR product from the 18S PCR assay was purified and consequently quantified and was further 10-fold diluted to achieve the sensitivity of the LAMP assay (Fig. S1a). The sensitivity of the LAMP assay was estimated to be up to  $10^{-6}$  dilution limit using the amplified and purified DNA having average concentration of 648.6 ng/ul and 550.2 ng/ul for *H. canis* and *E. canis*, respectively.  $10^{-6}$  dilution limit of the dilution series (Fig. S1b) was observed to be the limit of detection for the LAMP assay on the basis of the ladder-like pattern observed in the agarose gel electrophoresis images. The same tubes



**Fig. 2** White LED light (a) and ultraviolet (UV) LED light, (b) for viewing the sample results after the LAMP assay

were observed under UV and showed the same results with detection limit of  $10^{-6}$  dilution (Fig. S1b).

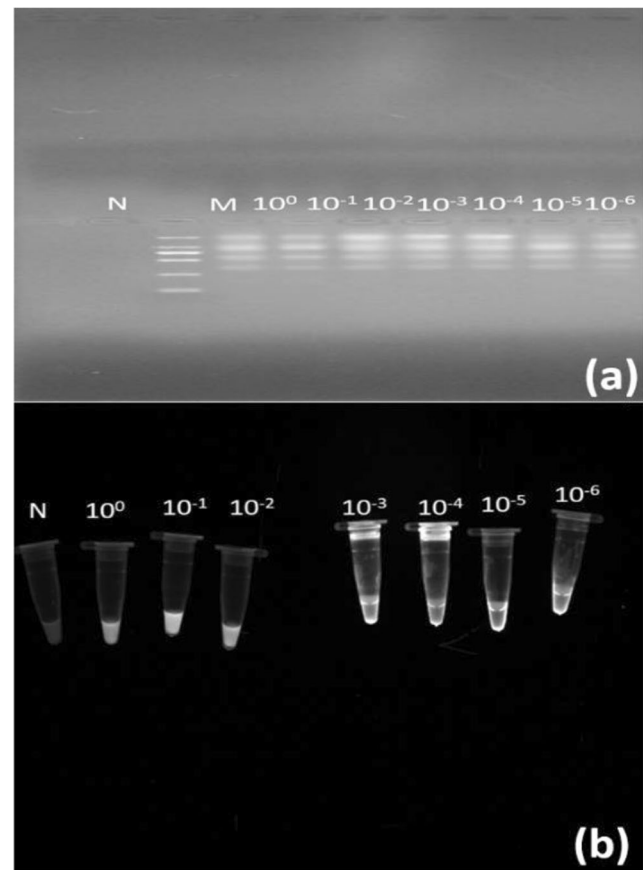
Moreover, no cross amplification was seen after the addition of the positive DNA templates of *B. canis vogeli*, as evident from the agarose gel electrophoresis (Fig. S2a) and simultaneously observed under UV light (Fig. S2b).

Following the incubation step of the LAMP assay, the amplified DNA contained in a reaction tube resulting from the LAMP assay was visualized by two observers by naked eyes, who were alternatively blinded to the setup of the assay, during and after the entire experiment (Fig. S3). Pinkish purple (dark blue to light blue on long-term/short-term storage of the LAMP products) corresponded to a positive reaction (presence of amplification) and no color change or transparent as a negative reaction.

The diagnostic capacity of the smartphone-assisted portable LAMP box as a rapid and efficient diagnostic tool was addressed by testing the previously collected and identified genomic DNA (unpublished data) using the LAMP box, which revealed that 5.3% (12/226) and 15.0% (34/226) of samples were positive for *H. canis* and *E. canis*, respectively, which clearly matched with the previously collected data for the same samples. The results were in agreement with the observed results. The LAMP assay was statistically checked for its sensitivity, and the corresponding values were represented at 95% confidence intervals (CI), and the observed sensitivity was around 96.4% falling within a range of 89.5–100.0% using the SPSS V.17.0 program. All the data of the LAMP assay was found to be statistically significant at  $p < 0.01$ .

In this study, we developed a lucid, comprehensible, economic, competitive, and a mobile LAMP detection system that utilizes the rigorous and robust LAMP assay for canine parasitic vector-borne infectious diseases and the flexibility and adaptability of smartphones to facilitate in-field POC diagnostics (Fig. 3).

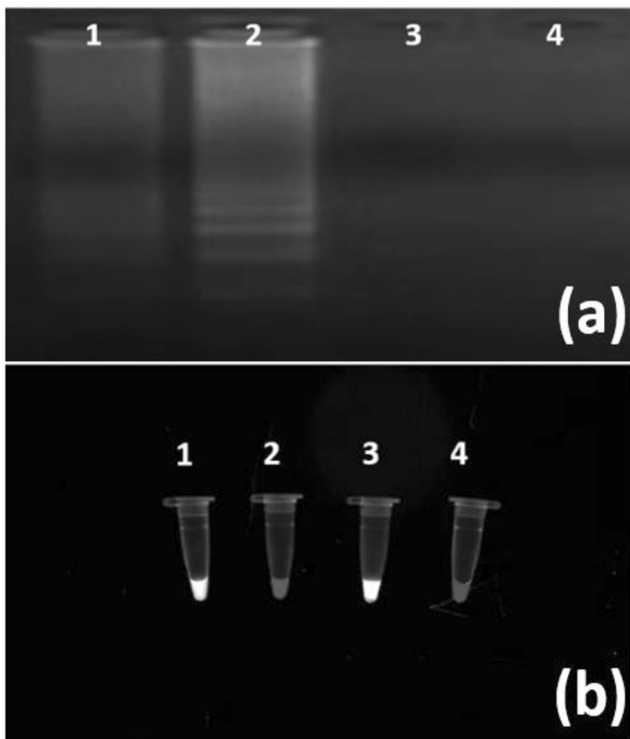
The comprehensive NAAT device comprised the following primary modules: (A) isothermal heating pad, (B) LED white light, (C) LED UV light, (D) heat sensor, (E) Arduino esp8266, and (F) a smartphone (Fig. 3). The isothermal heating pad could accommodate 5–6 0.5 ml Eppendorf reaction tubes, and hence, 5–6 samples can be processed at a time. Thermal parameters provided in the thermal cyclers or PCR machines which are sold by high-end reputed companies and brands are often tricky and intricate due to their bulky nature and huge thermal blocks. Distinctively, the isothermal nucleic acid amplification devices use less complicated thermal platforms. For instance, in this platform, we employed a small isothermal heating pad that was operated by using an externally available power source of 5 V. The heater that we used utilized comparatively lesser energy which was fulfilling its need to provide and maintain a consistent surface temperature profile for 45 min. Currently, most of the thermal cyclers are using silver blocks to facilitate heat resistant and uninterrupted fluctuations in the temperatures. Here, we used a very simple and handy miniature



**Fig. 3** Schematic representation of the portable LAMP box settings. A Isothermal heating pad, B LED white light, C UV LED light, D heat sensor, E Arduino esp8266, and F camera of any smartphone

aluminum block with grooves which could easily withstand high temperatures and fluctuations. Also, isothermal heaters often require modified PCR tubes. We tried to keep the experimental profile simple and easily available by using the conventionally used PCR tubes used in laboratory to process the LAMP tests. The isothermal heating pad, LED lights (UV and white), and heat sensor were initialized wirelessly through the webpage opened in the smartphone (router: 192.168.4.1). This was done through the Arduino esp8266 (Fig. 4a), which when connected, permitted the user end to recognize and set up a Wi-Fi connection named HanVet through the control screen panel (Fig. 4b). It directed it to a screen which had three tabs on the main screen (i) Run test tab, (ii) UV On tab, and (iii) UV Off tab (Fig. 4c).

Once the router or the webpage was launched, the control panel screen permitted the end-point user to set up a connection with the LAMP box, managed the heater temperature, and monitored the thermal profile on real-time basis as an ongoing process which could be easily viewed on the screen of the smartphone. The LAMP box was covered using a printed black paper to facilitate unblocked viewing of the end-point results and to provide consistent LED illumination. The unique features of this smartphone-enabled LAMP box are its portability and its usage in field applications.



**Fig. 4** **a** Mobile screen showing the router address for launching the webpage for the LAMP set up, **b** Wi-Fi connection HanVet establishment through the control screen panel, and **c** GUI page for the LAMP setup

## Discussion

Here, we demonstrate the idea of a point-of-care testing system for canine parasitic vector-borne diseases, utilizing any conventional smartphone that couples with the LAMP assay to facilitate mobile detection of diseases of zoonotic concern. The nucleic acid tests (NAT) serve as a valuable, much reliable, and advanced diagnostic tool for the detection and identification of pathogens, in combination with clinical findings and serological tests. This smartphone-enabled LAMP box contains the entire setup for processing the LAMP reaction and further visualizing the results. This entire setup is connected to our smartphone via Wi-Fi. All the software programming has been done using C language. This LAMP box is a portable, cost-effective diagnostic platform for diagnosis of several vector-borne diseases of the canine populations.

Although many technologies using the smartphone and LAMP assay have been previously developed, like the smartphone-based detection assay for Zika, chikungunya, and dengue viruses using a multiplex platform and quenching techniques for accurate detection in a multiplex system (Teoh et al. 2013; Priye et al. 2017), but these involve an expensive setup and quenching dyes for differentiating between different targets in a closed system. On the contrary, our smartphone-enabled portable LAMP assay is an economical, user-friendly, and specific assay for the detection of canine infectious diseases. It does not require

the need of additional quenching dyes; it just requires the addition of a fluorescent detection dye for the viewing of a positive sample. Our developed assay serves as a qualitative detection platform, which definitely has a scope for modifications and further studies in terms of advancing the technology. In a similar such study, a research group developed an uncomplicated, reasonable, water-activated, chemically heated, minimally instrumented smart cup for nucleic acid amplification and detection targeting the herpes simplex virus type 2 (HSV-2) (Liao et al. 2016). While the routine PCR tests rely on dedicated and special need specialized equipment or machinery, LAMP assays can be carried out in a simple heating block/water bath, knocking out the specific requirements for thermocyclers, and they are validated and standardized for direct visualization of results by naked eye, getting rid of the need for agarose gel electrophoresis (Notomi et al. 2000). This can ultimately lead to the reduced cost and ease for setting up a budget NAT diagnostic test for a veterinary clinic and related services. More importantly, with fewer modifications and validations in the LAMP assay, the overall turnaround time of the test can be drastically reduced without compromising on the quality of the experiment and results (Parida et al. 2008). Microscopy is a classical technique which has been continuously used since long time, for the identification and detection of hemoparasites. Conveniently, detection and visualizing of the circulating gamonts in stained blood smears is the most common approach used to check the presence of *H. canis* infection. The absence of infection is not always indicated by the absence of parasitemia, because there is a possibility of false-negative results in dogs having tissue infection with *Hepatozoon* spp. irrespective of the lower or a temporary state of parasitemia. PCR tests and similar amplification methods are considered to be a gold standard for the detection of even smaller amounts of the parasitic DNA and pathogens, since it becomes difficult to detect the lower parasitemia by the traditional microscopy techniques. In the present study, a specific LAMP assay on the basis of the partial 18S rRNA gene and p30 gene of *H. canis* and *E. canis* was optimized for the rapid detection of these infections in canine populations. The LAMP assay was found to be specific and did not amplify the DNA of *B. canis vogeli*, negative sample, and no-template controls displaying a characteristic ladder-like patterns. The developed LAMP assay can be conveniently used field for regular testing, when the high-end and state-of-the-art equipment are not accessible.

The rising canine population on a global scenario can be threatening with respect to the animal welfare and public health, as dogs serve as significant reservoirs of many zoonotic parasites and diseases. Doxycycline is one of the antibiotic drugs that is popularly used to treat dogs suffering from *E. canis* infections and related pathogens (Bowman 2011). Profound and large-scale usage of this particular antibiotic for treating the infections in dogs can give rise to an alarming situation, by giving rise to the resistant strains (Harrus and Waner 2011). The clinical signs observed in the canine diseases are very much inter-related and overlapping



with the symptoms of several other diseases occurring in dogs, and it becomes very crucial and important to use both serological tests and NAT to reach a definite conclusion related to the diagnosis of a particular canine disease (Harrus and Waner 2011). This constructive approach towards the diagnosis of canine parasitic diseases will not only allow the veterinary practitioners to suggest appropriate treatments but will also help them to modify the existing treatment systems (Harrus and Waner 2011). LAMP assay has been popularly and effectively used for the diagnosis of a wide range of pathogens and infectious diseases, including canine pathogens and diseases (Stich et al. 2002; Harrus et al., 2011; Faggion et al. 2013). In this respect, LAMP assay and technique can be very helpful for the overall management and treatment of the canine infectious diseases, specifically in tropical and subtropical areas where such diseases occur more prevalently and where the veterinary laboratories often have negligible or limited access to advanced and modern equipment. Isothermal amplification techniques have a great potential over the traditional gold standard methods like PCR. An important point to be considered for the existing LAMP-based POC platforms in resource-limited settings is ultimately the final cost of the test and the reaction components. Indeed, the existing and the upcoming molecular technologies come with a higher turnaround time, sophisticated and power-consuming modern equipment, and undoubtedly the need for skilled labor. Smarter amalgamation by using smart and mobile devices is a key to the modifications in the existing LAMP-based devices.

## Conclusion

Isothermal amplification techniques especially LAMP is a trustworthy and reliable alternative to the traditional gold standard, PCR for the diagnosis and detection of pathogens in the canine populations. We have developed an economical and easy to handle smartphone-assisted portable LAMP box altogether connected to each other wirelessly which facilitates the diagnosis of the parasitic diseases in canine population powered by a 5 V power bank or power source eliminating the need of any external electricity source. The sensitivity limit of the LAMP assay was found to be up to  $10^{-6}$  dilution limit using the amplified specific DNA templates, and around 5–6 samples could be tested at one time. The use of this diagnostic tool is not only limited to the diagnosis of various important canine vector-borne diseases like *E. canis* and *H. canis* but can also have a wide applicability in the field of other parasitic vector-borne infectious diseases diagnosis including those of zoonotic origin.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00436-021-07077-z>.

**Acknowledgements** We would like to express our gratitude towards Zihe Wang and Aslam Niaz Khan for their helpful suggestions and for procurement of project components.

**Author contribution** All the authors read, revised, and approved the submitted version.

**Funding** This work was funded by the National Key R&D Program of China (2016YFC1201602) and Hainan Provincial Key R&D Programs (ZDYF2019073; ZDYF2018085).

## Declarations

**Ethics approval** The care and use of animals in this study were approved by the Hainan University Institutional Animal Care and Use Committee. We also confirmed that all experiments were performed in accordance with institutional guidelines and regulations.

**Conflict of interest** The authors declare no competing interests.

**Patent** We are in the process of preparations to apply for a patent for our smartphone-assisted portable LAMP box.

## References

- Abd Rani PA, Irwin PJ, Coleman GT, Gatne M, Traub RJ (2011) A survey of canine tick-borne diseases in India. *Parasit Vectors* 4: 141. Published 2011 Jul 19. <https://doi.org/10.1186/1756-3305-4-141>
- Abdul-Ghani R, Al-Mekhlafi AM, Karanis P (2012) Loop-mediated isothermal amplification (LAMP) for malarial parasites of humans: would it come to clinical reality as a point-of-care test? *Acta Trop* 122(3):233–240. <https://doi.org/10.1016/j.actatropica.2012.02.004>
- Ahmed MU, Hossain MM, Tamiya E (2008) Electrochemical biosensors for medical and food applications. *Electro Anal* 20(6):616–626
- Aktas M, Özübek S, Altay K, Balkaya İ, Utuk AE, Kırbas A, Şimsek S, Dumanlı N (2015) A molecular and parasitological survey of *Hepatozoon canis* in domestic dogs in Turkey. *Vet Parasitol* 209(3–4):264–267. <https://doi.org/10.1016/j.vetpar.2015.02.015>
- Al Izzi S, Martin DS, Chan RY, Leutenegger CM (2013) *Babesia canis vogeli*, *Ehrlichia canis*, and *Anaplasma platys* infection in a dog. *Vet Clin Pathol* 42(4):471–475. <https://doi.org/10.1111/vcp.12090>
- Alhassan A, Thekisoe OM, Yokoyama N et al (2007) Development of loop-mediated isothermal amplification (LAMP) method for diagnosis of equine piroplasmiasis. *Vet Parasitol* 143(2):155–160. <https://doi.org/10.1016/j.vetpar.2006.08.014>
- Ali MM, Li F, Zhang Z, Zhang K, Kang DK, Ankrum JA, le XC, Zhao W (2014) Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. *Chem Soc Rev* 43(10): 3324–3341. <https://doi.org/10.1039/c3cs60439j>
- Arora A, Simone G, Salieb-Beugelaar GB, Kim JT, Manz A (2010) Latest developments in micro total analysis systems. *Anal Chem* 82(12):4830–4847. <https://doi.org/10.1021/ac100969k>
- Asiello PJ, Baeumner AJ (2011) Miniaturized isothermal nucleic acid amplification, a review. *Lab Chip* 11(8):1420–1430. <https://doi.org/10.1039/c0lc00666a>
- Auroux PA, Iossifidis D, Reyes DR, Manz A (2002) Micro total analysis systems. 2. Analytical standard operations and applications. *Anal Chem* 74(12):2637–2652
- Baneth G, Shkap V (2003) Monozoic Cysts of *Hepatozoon canis*. *J Parasitol* 89(2):379–381



- Bowman DD (2011) Introduction to the alpha-proteobacteria: Wolbachia and Bartonella, Rickettsia, Brucella, Ehrlichia, and Anaplasma. *Top Companion Anim Med* 26(4):173–177. <https://doi.org/10.1053/j.tcam.2011.09.002>
- Cardozo GP, Santos EV, Fachin AL, Franca SC et al (2011) A glass bead protocol for recovery of host cell free Ehrlichia canis and quantification by Sybr-green real-time PCR. *Biocell* 35:35–36
- Chang CM, Chang WH, Wang CH, Wang JH, Mai JD, Lee GB (2013) Nucleic acid amplification using microfluidic systems. *Lab Chip* 13(7):1225–1242. <https://doi.org/10.1039/c3lc41097h>
- Chin CD, Linder V, Sia SK (2012) Commercialization of microfluidic point-of-care diagnostic devices. *Lab Chip* 12(12):2118–2134. <https://doi.org/10.1039/c2lc21204h>
- Compton J (1991) Nucleic acid sequence-based amplification. *Nature* 350(6313):91–92. <https://doi.org/10.1038/350091a0>
- Craw P, Balachandran W (2012) Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. *Lab Chip* 12(14):2469–2486. <https://doi.org/10.1039/c2lc40100b>
- De Tommasi AS, Otranto D, Dantas-Torres F, Capelli G, Breitschwerdt EB, de Caprariis D (2013) Are vector-borne pathogen co-infections complicating the clinical presentation in dogs? *Parasit Vectors* 6:97. Published 2013 Apr 15. <https://doi.org/10.1186/1756-3305-6-97>
- Doyle CK, Labruna MB, Breitschwerdt EB, Tang YW, Corstvet RE, Hegarty BC, Bloch KC, Li P, Walker DH, McBride JW (2005) Detection of medically important Ehrlichia by quantitative multicolor TaqMan real-time polymerase chain reaction of the dsb gene. *J Mol Diagn* 7(4):504–510. [https://doi.org/10.1016/S1525-1578\(10\)60581-8](https://doi.org/10.1016/S1525-1578(10)60581-8)
- Easley CJ, Karlinsky JM, Bienvenue JM, Legendre LA, Roper MG, Feldman SH, Hughes MA, Hewlett EL, Merkel TJ, Ferrance JP, Landers JP (2006) A fully integrated microfluidic genetic analysis system with sample-in-answer-out capability. *Proc Natl Acad Sci U S A* 103(51):19272–19277. <https://doi.org/10.1073/pnas.0604663103>
- Eiras DF, Craviotto MB, Vezzani D, Eyal O, Baneth G (2013) First description of natural Ehrlichia canis and Anaplasma platys infections in dogs from Argentina. *Comp Immunol Microbiol Infect Dis* 36(2):169–173. <https://doi.org/10.1016/j.cimid.2012.11.008>
- Faggion S, Salvador A, Jacobino K, Bortolotto L, Lopes MB, Silva M, Santos EV, Fachin AL, Franca SC, Marins M (2013) Loop-mediated isothermal amplification assay for the detection of Ehrlichia canis DNA in blood samples from dogs. *Arch Med Vet* 45:197–201
- Gauglitz G (2014) Point-of-Care Platforms. *Annu Rev Anal Chem* 7:297–315
- Gavazza A, Bizzeti M, Papini R (2003) Observations on dogs found naturally infected with Hepatozoon canis in Italy. *Rev Med Vet* 154:565–571
- Hall MJ, Wharam SD, Weston A, Cardy DL, Wilson WH (2002) Use of signal-mediated amplification of RNA technology (SMART) to detect marine cyanophage DNA. *Biotechniques*. 32(3):604–611. <https://doi.org/10.2144/02323rr02>
- Harrus S, Waner T (2011) Diagnosis of canine monocytotropic ehrlichiosis (Ehrlichia canis): an overview. *Vet J* 187(3):292–296. <https://doi.org/10.1016/j.tvjl.2010.02.001>
- He L, Zhou YQ, Oosthuizen MC, Zhao JL (2009) Loop-mediated isothermal amplification (LAMP) detection of Babesia orientalis in water buffalo (Bubalus bubalis, Linnaeus, 1758) in China. *Vet Parasitol* 165(1–2):36–40. <https://doi.org/10.1016/j.vetpar.2009.06.036>
- Ikadai H, Tanaka H, Shibahara N, Matsuu A, Uechi M, Itoh N, Oshiro S, Kudo N, Igarashi I, Oyamada T (2004) Molecular evidence of infections with Babesia gibsoni parasites in Japan and evaluation of the diagnostic potential of a loop-mediated isothermal amplification method. *J Clin Microbiol* 42(6):2465–2469. <https://doi.org/10.1128/JCM.42.6.2465-2469.2004>
- Inokuma H, Okuda M, Ohno K, Shimoda K, Onishi T (2002) Analysis of the 18S rRNA gene sequence of a Hepatozoon detected in two Japanese dogs. *Vet Parasitol* 106(3):265–271. [https://doi.org/10.1016/S0304-4017\(02\)00065-1](https://doi.org/10.1016/S0304-4017(02)00065-1)
- Iwamoto T, Sonobe T, Hayashi K (2003) Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. *J Clin Microbiol* 41(6):2616–2622. <https://doi.org/10.1128/jcm.41.6.2616-2622.2003>
- Jani IV, Peter TF (2013) How point-of-care testing could drive innovation in global health. *N Engl J Med* 368(24):2319–2324. <https://doi.org/10.1056/NEJMs1214197>
- Jiang L, Mancuso M, Lu Z, Akar G, Cesarman E, Erickson D (2014) Solar thermal polymerase chain reaction for smartphone-assisted molecular diagnostics. *Sci Rep* 4:4137. Published 2014 Feb 20. <https://doi.org/10.1038/srep04137>
- Kaneko H, Kawana T, Fukushima E, Suzutani T (2007) Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. *J Biochem Biophys Methods* 70(3):499–501. <https://doi.org/10.1016/j.jbbm.2006.08.008>
- Karanis P, Ongerth J (2009) LAMP—a powerful and flexible tool for monitoring microbial pathogens. *Trends Parasitol* 25(11):498–499. <https://doi.org/10.1016/j.pt.2009.07.010>
- Karanis P, Thekisoe O, Kioptsis K, Ongerth J, Igarashi I, Inoue N (2007) Development and preliminary evaluation of a loop-mediated isothermal amplification procedure for sensitive detection of cryptosporidium oocysts in fecal and water samples. *Appl Environ Microbiol* 73(17):5660–5662. <https://doi.org/10.1128/AEM.01152-07>
- Kiddle G, Hardinge P, Buttigieg N, Gandelman O, Pereira C, McElgunn CJ, Rizzoli M, Jackson R, Appleton N, Moore C, Tisi LC, Murray JA (2012) GMO detection using a bioluminescent real time reporter (BART) of loop mediated isothermal amplification (LAMP) suitable for field use. *BMC Biotechnol* 12:15. <https://doi.org/10.1186/1472-6750-12-15>
- Kledmanee K, Suwanpakdee S, Krajangwong S et al (2009) Development of multiplex polymerase chain reaction for detection of Ehrlichia canis, Babesia spp and Hepatozoon canis in canine blood. *Southeast Asian J Trop Med Public Health* 40(1):35–39
- Kovarik ML, Ormoff DM, Melvin AT, Dobes NC, Wang Y, Dickinson AJ, Gach PC, Shah PK, Allbritton NL (2013) Micro total analysis systems: fundamental advances and applications in the laboratory, clinic, and field. *Anal Chem* 85(2):451–472. <https://doi.org/10.1021/ac3031543>
- Krasteva D, Toubiana M, Hartati S, Kusumawati A, Dubremetz JF, Widada JS (2009 Jun) Development of loop-mediated isothermal amplification (LAMP) as a diagnostic tool of toxoplasmosis. *Vet Parasitol* 162(3–4):327–331. <https://doi.org/10.1016/j.vetpar.2009.03.014>
- Latrofa MS, Dantas-Torres F, Giannelli A, Otranto D (2014) Molecular detection of tick-borne pathogens in Rhipicephalus sanguineus group ticks. *Ticks Tick-Borne Dis* 5:943–946
- Lau YL, Lai MY, Teoh BT et al (2015) Colorimetric Detection of dengue by single tube reverse-transcription-loop-mediated isothermal amplification. *PLoS One* 10(9):e0138694. Published 2015 Sep 18. <https://doi.org/10.1371/journal.pone.0138694>
- Liao SC, Peng J, Mauk MG, Awasthi S, Song J, Friedman H, Bau HH, Liu C (2016) Smart cup: a minimally-instrumented, smartphone-based point-of-care molecular diagnostic device. *Sensors Actuators B Chem* 229:232–238. <https://doi.org/10.1016/j.snb.2016.01.073>
- Lui C, Cady NC, Batt CA (2009) Nucleic acid-based detection of bacterial pathogens using integrated microfluidic platform systems. *Sensors (Basel)* 9(5):3713–3744. <https://doi.org/10.3390/s90503713>

- Ma C, Han D, Deng M, Wang J, Shi C (2015) Single primer-triggered isothermal amplification for double-stranded DNA detection. *Chem Commun (Camb)* 51(3):553–556. <https://doi.org/10.1039/c4cc07845d>
- Mandal M, Banerjee PS, Kumar S, Ram H, Garg R, Pawde AM (2015) Development of loop-mediated isothermal amplification (LAMP) for detection of *Babesia gibsoni* infection in dogs. *Vet Parasitol* 209(1–2):50–55. <https://doi.org/10.1016/j.vetpar.2015.02.008>
- McBride JW, Corstvet RE, Gaunt SD, Chinsangaram J, Akita GY, Osburn BI (1996) PCR detection of acute *Ehrlichia canis* infection in dogs. *J Vet Diagn Investig* 8(4):441–447. <https://doi.org/10.1177/104063879600800406>
- Mekuzas Y, Gradoni L, Oliva G, Foglia Manzillo V, Baneth G (2009) *Ehrlichia canis* and *Leishmania infantum* co-infection: a 3-year longitudinal study in naturally exposed dogs. *Clin Microbiol Infect* 15(Suppl 2):30–31. <https://doi.org/10.1111/j.1469-0691.2008.02150.x>
- Mori Y, Nagamine K, Tomita N, Notomi T (2001) Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. *Biochem Biophys Res Commun* 289(1):150–154. <https://doi.org/10.1006/bbrc.2001.5921>
- Mylonakis ME, Leontides L, Gonen L, Billinis C, Koutinas AF, Baneth G (2005) Anti-*Hepatozoon canis* serum antibodies and gamonts in naturally-occurring canine monocytic ehrlichiosis. *Vet Parasitol* 129:229–233
- Nakaghi AC, Machado RZ, Ferro JA, Labruna MB et al (2010) Sensitivity evaluation of a single-step PCR assay using *Ehrlichia canis* p28 gene as a target and its application in diagnosis of canine ehrlichiosis. *Rev Bras Parasitol Vet* 19:75–79
- Nava S, Estrada-Peña A, Petney T, Beati L, Labruna MB, Szabó MPJ, Venzal JM, Mastropaolo M, Mangold AJ, Guglielmone AA (2015) The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). *Vet Parasitol* 208(1–2):2–8. <https://doi.org/10.1016/j.vetpar.2014.12.021>
- 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>
- Notomi T, Mori Y, Tomita N, Kanda H (2015) Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *J Microbiol* 53:1–15. <https://doi.org/10.1007/s12275-015-4656-9>
- Otranto D, Dantas-Torres F (2010) Canine and feline vector-borne diseases in Italy: current situation and perspectives. *Parasit Vectors* 3:2. Published 2010 Jan 11. <https://doi.org/10.1186/1756-3305-3-2>
- Otranto D et al (2011) Diagnosis of *Hepatozoon canis* in young dogs by cytology and PCR. *Parasit Vectors* 4:55. <https://doi.org/10.1186/1756-3305-4-55>
- Oyhenart J, Martinez F, Ramirez R, Fort M, Breccia JD (2013) Loop mediated isothermal amplification of 5.8S rDNA for specific detection of *Trichomonas foetus*. *Vet Parasitol* 193:59–65
- Parida M, Sannarangaiah S, Dash PK, Rao PV, Morita K (2008) Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. *Rev Med Virol* 18(6):407–421. <https://doi.org/10.1002/rmv.593>
- Peleg O, Baneth G, Eyal O, Inbar J, Harrus S (2010) Multiplex real-time qPCR for the detection of *Ehrlichia canis* and *Babesia canis vogeli*. *Vet Parasitol* 173(3–4):292–299. <https://doi.org/10.1016/j.vetpar.2010.06.039>
- Pinhanelli VC, Costa PN, Silva G et al (2015) Development and evaluation of a loop-mediated isothermal amplification assay for detection of *Ehrlichia canis* DNA in naturally infected dogs using the p30 gene. *Genet Mol Res* 14(4):17885–17892. Published 2015 Dec 22. <https://doi.org/10.4238/2015.December.22.13>
- Priye A & Ugaz VM (2016) In Microfluidic methods for molecular biology 55–69 (Springer)
- Priye A, Bird SW, Light YK, Ball CS, Negrete OA, Meagher RJ (2017) A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Sci Rep* 7:44778. Published 2017 Mar 20. <https://doi.org/10.1038/srep44778>
- Sackmann EK, Fulton AL, Beebe DJ (2014) The present and future role of microfluidics in biomedical research. *Nature*. 507(7491):181–189. <https://doi.org/10.1038/nature13118>
- Santos F, Coppede JS, Pereira AL et al (2009) Molecular evaluation of the incidence of *Ehrlichia canis*, *Anaplasma platys* and *Babesia* spp. in dogs from Ribeirão Preto, Brazil. *Vet J* 179(1):145–148. <https://doi.org/10.1016/j.tvjl.2007.08.017>
- Seyrig G, Ahmad F, Stedtfeld RD, Tournouse DM, Hashsham SA (2011) Simple, Powerful, and smart: using LAMP for lowcost screening of multiple waterborne pathogens. In: *Environmental microbiology: current technology and water applications*. Caister Academic, Cambridge, pp 103–125
- Shi C, Liu Q, Ma C, Zhong W (2014) Exponential strand-displacement amplification for detection of microRNAs. *Anal Chem* 86(1):336–339. <https://doi.org/10.1021/ac40388043>
- Shkap V, Baneth G, Pipano E (1994) Circulating antibodies to *Hepatozoon canis* demonstrated by immunofluorescence. *J Vet Diagn Investig* 6(1):121–123. <https://doi.org/10.1177/104063879400600127>
- Singh K, Singh H, Singh NK, Kashyap N, Sood NK, Rath SS (2017a) Molecular prevalence, risk factors assessment and haemato-biochemical alterations in hepatozoonosis in dogs from Punjab, India. *Comp Immunol Microbiol Infect Dis* 55:53–58. <https://doi.org/10.1016/j.cimid.2017.10.001>
- Singh KP, Singh H, Singh ND, Kashyap N, Rath SS (2017b) Molecular prevalence of *Hepatozoon canis* infection in dog tick, *Rhipicephalus sanguineus*, from Punjab, India. *J Anim Res* 7:401
- Singh MD, Singh H, Singh NK, Singh NK, Kashyap N, Sood NK, Rath SS (2019) Development of loop-mediated isothermal amplification (LAMP) assay for detection of *Hepatozoon canis* infection in dogs. *Ticks Tick Borne Dis* 10(2):371–376. <https://doi.org/10.1016/j.ttbdis.2018.11.016>
- Song J, Mauk MG, Hackett BA, Cherry S, Bau HH, Liu C (2016) Instrument-free point-of-care molecular detection of Zika virus [published correction appears in *Anal Chem*. 2018 Nov 20;90(22):13814]. *Anal Chem* 88(14):7289–7294. <https://doi.org/10.1021/acs.analchem.6b01632>
- Stich RW, Rikihisa Y, Ewing SA, Needham GR, Grover DL, Jittapalpong S (2002) Detection of *Ehrlichia canis* in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay. *J Clin Microbiol* 40(2):540–546. <https://doi.org/10.1128/jcm.40.2.540-546.2002>
- Tanner NA, Evans TC Jr (2014) Loop-mediated isothermal amplification for detection of nucleic acids. *Curr Protoc Mol Biol* 105:Unit 15.14 Published 2014 Jan 6. <https://doi.org/10.1002/0471142727.mb1514s105>
- Teoh B, Sam S, Tan K et al (2013) Detection of dengue viruses using reverse transcription-loop-mediated isothermal amplification. *BMC Infect Dis* 13:387. <https://doi.org/10.1186/1471-2334-13-387>
- Thekisoe OM, Inoue N, Kuboki N et al (2005) Evaluation of loop-mediated isothermal amplification (LAMP), PCR and parasitological tests for detection of *Trypanosoma evansi* in experimentally infected pigs. *Vet Parasitol* 130(3–4):327–330. <https://doi.org/10.1016/j.vetpar.2005.04.019>
- Thekisoe OM, Rambritch NE, Nakao R, Bazie RS, Mbatia P, Namangala B, Malele I, Skilton RA, Jongejan F, Sugimoto C, Kawazu S, Inoue N (2010) Loop-mediated isothermal amplification (LAMP) assays for detection of *Theileria parva* infections targeting the PIM and p150 genes. *Int J Parasitol* 40(1):55–61. <https://doi.org/10.1016/j.ijpara.2009.07.004>
- Tian B, Qiu Z, Ma J, Zardán Gómez de la Torre T, Johansson C, Svedlindh P, Strömberg M (2016) Attomolar Zika virus

- oligonucleotide detection based on loop-mediated isothermal amplification and AC susceptometry. *Biosens Bioelectron* 86:420–425. <https://doi.org/10.1016/j.bios.2016.06.085>
- Toumazou C, Shepherd LM, Reed SC, Chen GI, Patel A, Garner DM, Wang C-JA, Ou C-P, Amin-Desai K, Athanasiou P, Bai H, Brizido IMQ, Caldwell B, CoomberAlford D, Georgiou P, Jordan KS, Joyce JC, La Mura M, Morley D, Sathyavathan S, Temelso S, Thomas RE, Zhang L (2013) Simultaneous DNA amplification and detection using a pH-sensing semiconductor system. *Nat Methods* 10:641–646
- Vashist SK, Mudanyali O, Schneider EM, Zengerle R, Ozcan A (2014) Cellphone-based devices for bioanalytical sciences. *Anal Bioanal Chem* 406(14):3263–3277. <https://doi.org/10.1007/s00216-013-7473-1>
- Vashist SK, Luppia PB, Yeo LY, Ozcan A, Luong JHT (2015) Emerging technologies for next-generation point-of-care testing. *Trends Biotechnol* 33(11):692–705. <https://doi.org/10.1016/j.tibtech.2015.09.001>
- Vincent M, Xu Y, Kong H (2004) Helicase-dependent isothermal DNA amplification. *EMBO Rep* 5(8):795–800. <https://doi.org/10.1038/sj.embor.7400200>
- Wang L, Shi L, Alam MJ, Geng Y, Li L (2008) Specific and rapid detection of foodborne Salmonella by loop-mediated isothermal amplification method. *Food Res Int* 41(1):69–74
- Wang LX, He L, Fang R, Song QQ, Tu P, Jenkins A, Zhou YQ, Zhao JL (2010) Loop-mediated isothermal amplification (LAMP) assay for detection of *Theileria sergenti* infection targeting the p33 gene. *Vet Parasitol* 171:159–162
- Xu G, Hu L, Zhong H, Wang H, Yusa SI, Weiss TC, Romaniuk PJ, Pickerill S, You Q (2012) Cross priming amplification: mechanism and optimization for isothermal DNA amplification. *Sci Rep* 2:246. <https://doi.org/10.1038/srep00246>
- Yager P, Domingo GJ, Gerdes J (2008) Point-of-care diagnostics for global health. *Annu Rev Biomed Eng* 10:107–144. <https://doi.org/10.1146/annurev.bioeng.10.061807.160524>
- Yan L, Zhou J, Zheng Y, Gamson AS, Roembke BT, Nakayama S, Sintim HO (2014) Isothermal amplified detection of DNA and RNA. *Mol BioSyst* 10(5):970–1003. <https://doi.org/10.1039/c3mb70304e>
- Yang S, Rothman RE (2004) PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis* 4(6):337–348. [https://doi.org/10.1016/S1473-3099\(04\)01044-8](https://doi.org/10.1016/S1473-3099(04)01044-8)
- Zhang X, Lowe SB, Gooding JJ (2014) Brief review of monitoring methods for loop-mediated isothermal amplification (LAMP). *Biosens Bioelectron* 61:491–499. <https://doi.org/10.1016/j.bios.2014.05.039>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.