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Letter to the Editor

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Loop-mediated isothermal amplification in the field: reality beyond the potentiality

Donato Antonio RAELE*, **Nicola PUGLIESE**, **Domenico GALANTE**, **Maria Assunta CAFIERO**
Experimental Zoophophylactic Institute of Puglia and Basilicata, Foggia, Italy

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To the Editor,

Loop-mediated isothermal amplification (LAMP) is a very promising technique for DNA amplification, first described by Notomi et al. [1] and summarized by Raele et al. [2]. It mainly relies on the action of a DNA polymerase that, beyond the common 5'-3' DNA synthesis, has strand displacement activity. The final product of LAMP is a series of concatemers, whose repeating units are the replicates of the selected DNA target. The specificity is granted by the six different sites to be recognized on the template to prime the reaction, six different sites have to be recognized by the primers on the template [3], and, from a more practical perspective, LAMP has benefits of simplicity and rapidity, as the reaction may be carried out at a constant temperature, without need of thermal cycles (and thermal cycler as well), and it takes about 40–50 min.

Because of those features, LAMP has been suggested by many authors as suitable for use in the field and on the spot. Among them, Centeno-Cuadros et al. set up a LAMP protocol [4] suitable for the sex determination of three raptor species in the field, and more recently Lee [5] pointed out the suitability of the LAMP approach for a wider range of in-field applications, including the detection of pathogens and parasites in the environment for monitoring purposes.

Despite its potential, the actual application of LAMP in the field has been hampered by some technical challenges. Among them, there is the need to expose the reaction to UV rays to detect positivity after addition of fluorescent intercalating dyes. Another problem is the unavailability of handily portable thermo-blocks. Only recently, new products have been devised to make it possible to carry out LAMP outside of laboratories. In particular, portable instruments for the real-time detection of fluorescence have been introduced, and this may overcome the difficulties linked to the fluorescence detection by UV lamp, which needs a dark environment, often not possible to be achieved in the field.

To verify the actual feasibility of such a system in the field, we undertook a 1-day campaign aimed to apply LAMP to detect the presence of *Borrelia burgdorferi* s.l., or Lyme borrelia (LB), in ixodid ticks immediately after collection in the National Park of Gargano (Apulia, Italy).

Such a group of pathogens was chosen because of its clinical, epidemiological, and bacteriological relevance, and for being typical vector-borne pathogens, mostly transmitted to humans through the bite of the sheep tick *Ixodes ricinus* [6]. The location (the National Park of Gargano) was selected because of previous laboratory detection of *B. afzelii* from some pools of *I. ricinus* collected there (unpublished data).

The campaign was carried out by two investigators on a sunny day of June 2017, in order to find better conditions for the collection of ticks.

One of the investigators carried a light portable fridge, the other a small outdoor folding table; they both were also equipped with a backpack.

Backpacks contained:

- gloves,
- a 1-m^2 fabric made of white cotton flannel (for tick collection, TC),
- a 1.5-m wooden dowel (for TC),
- sterile forceps (for TC),
- sterile petri dishes (for TC)
- sterile plastic tubes filled with 70% ethanol (for tick storage),
- a portable stereomicroscope (for tick identification),
- a pipette set (P10, P200, P1000) with respective sterile filter tips,
- sterile 1.5-mL microcentrifuge tubes (for DNA extraction, DE),
- sterile pestles and mortars (for DE),
- Plant Material DNA extraction kit (Optigene, Horsham, UK) (for DE),
- a portable Genie III instrument (Optigene) (for LAMP reaction, LR),

* Correspondence: donatoantonio.raele@izspb.it

- autoclavable plastic bags for wastes;

while in the portable fridge there were:

- sterile 0.2-mL tubes for qPCR (for LR),

- two cooling blocks for vials and 0.2-mL tubes, respectively (for LR),

- an aliquot of LAMP reaction mix in a vial placed in the cooling block (for LR).

Free living ticks were harvested by using the flagging method, kept in ethanol 70% for 30 min, and identified at species level by morphological keys [7] with the aid of a portable stereomicroscope.

Out of the collected ticks, 5 were identified as *I. ricinus* nymphs, and they were used for DNA extraction. In order to disrupt the coriaceous dorsal shield of ixodid ticks, specimens were ground using sterile mortars and pestles (Figure 1) and DNA was recovered from the homogenate by using the Plant Material DNA extraction kit (Optigene, Horsham, UK), devised to extract DNA from the rigid structures of plants without the need of a centrifuge. The DNA solution (5 µL) was directly used as a template in LAMP reactions. They were set up in final volumes of 25 µL by using Isothemal Master Mix (Optigene), which contained all reagents with the exception of primers, and a ds-DNA binding fluorescent dye. The inner and outer primers [8] were added at a final concentration of 0.8 and 0.2 µM, respectively. An aliquot of DNA that was found to be positive for *B. afzelii* in our previous investigation was used as a positive control, and a reaction with sterile distilled water instead of DNA was used as a negative control. The seven reactions were simultaneously run in the portable instrument Genie III (Optigene), equipped with a dual-channel fluorescence detection system and a display that visualizes the fluorescence curves in real time (Figure 2). Furthermore, it was supplied with rechargeable batteries, released by the manufacturer as lasting 1 day. However, the device cannot be connected to the 12-V power supplies usually present in cars.

The system appeared to work efficiently. One of the five tested ticks was positive for *B. burgdorferi* s.l., and, the day after, the result was confirmed by PCR targeting the *groEL* gene [9]. The nucleotide sequence of the amplicons (GenBank accession numbers MF977273–MF977274) were both 100% identical to the corresponding sequences of *B. garinii* in GenBank.

On aggregate, the campaign evidenced the actual suitability of the LAMP approach for in-field molecular investigations. Two operators were able to accomplish a 1-day campaign aimed to achieve molecular results directly on the spot, and the volume and weight of the equipment are perfectly suitable for a such a small brigade. In the case of the present report, the campaign was aimed at the detection of a vector-borne pathogen, but it is clear that LAMP could be functional for a number of studies, as postulated by Wong et al. [10].

Figure 1. Grinding of a tick before DNA extraction in the field.

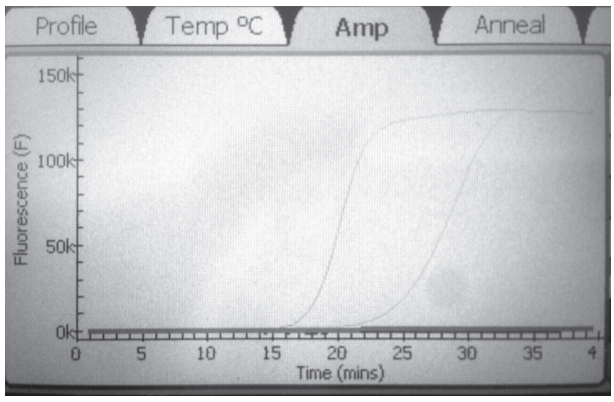

Figure 2. The display of the portable real-time instrument for LAMP reaction run and fluorescence detection.

However, procedures, and their respective costs, should be modulated in order to solve or circumvent some technical issues that could have hampered the actual concretization of such a kind of investigation. The first process to be considered is the nucleic acid extraction. The use of a simple and fast NaOH method [11] is a good choice whenever soft or easy-to-lyse materials have to be processed, but it was not appropriate to disrupt the chitin in the exoskeletons of arthropods. Therefore, we were forced to use an alternative protocol, suited for harder material. It should be underlined that the kit for plant material was not effective to grind a whole hard tick, so that a sterile pestle and mortar had to be used to bust the tick prior to the lysis. However, the practicality of the kit was increased by the fact that the DNA solution could be directly used as a template in LAMP reactions. The second, but not less critical, aspect to be taken into account is the LAMP system and, in particular, the visualization of the reaction. A very

simple and cheap solution is the addition of intercalating dyes to the reaction mix after the reaction. Despite the convenience of the system, the tubes have to be exposed to UV rays, so a UV lamp or even a UV transilluminator is needed. A potential alternative is malachite green, recently used to detect LAMP products [12]. It makes the UV detection unnecessary and good reproducibility was estimated in laboratory conditions. However, malachite green is hazardous for humans and the environment [13], and this should be carefully considered in the field, where safety equipment is not always available.

On the other hand, the adopted real-time system overcomes the practical difficulties in detecting the fluorescence by means of a portable UV lamp, and it reduces the use of potentially hazardous materials to the essential. The system has been designed for use in the field, despite the lack of an adapter for the 12-V power supplies of cars, which compels operators to rely on the battery.

In conclusion, in-field LAMP is no longer a conceptual or potential tool; it is destined to become a concrete strategy, which might provide useful and immediate information in a wide range of studies. However, pros and cons should be carefully balanced to assess whether benefits of an on-the-spot approach are worth an increase in costs.

Hopefully, in the near future, technological advances will make it even more affordable.

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