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Evaluation of different DNA Extraction Methods from Blood for LAMP Reaction

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ABSTRACT: DNA-based methods are becoming familiar techniques for the detection and identification of specific organisms. These methods rely on the ability to detect specific DNA sequences in the genome of target organisms. The main objective of the study is to facilitate DNA extraction from the blood at the field level. In the present study, simple methods of DNA extraction from blood were optimized and compared with kit method to assist LAMP (Loop mediated isothermal amplification). The boiling method was the simplest and cheapest when compared to all the four methods. But the quantity of DNA was highest in case of DPK (Detergent proteinase K) method. Apart from these methods, the filter paper method is also a simple, time saving method which can also be considered as a good alternative.

Keywords: DNA, LAMP, Boiling, DPK method, filter paper.

INTRODUCTION

DNA extraction has become an important step in most of the downstream applications. DNA-based methodologies have progressed impressively, being almost indispensable in most biology fields, including in disease diagnosis, forensic applications, and detection of several pathogenic microorganisms in clinical, food and environmental samples (Jobling and Gill 2004; Tang *et al*., 1997; Lockley and Bardsley 2000). In general, DNA extraction methods involve three main steps: cell disruption, DNA extraction and DNA purification. The boiling procedure is one of the simplest protocols, and largely used for total DNA extraction from microorganisms (Reischl *et al*., 2000; De Medici *et al*., 2003).

Whole blood samples are one of the main sources used to obtain DNA and there are many different protocols available in this issue. A highly efficient DNA extraction procedure from dried blood spots (DBS) onto Whatman 3MM filter paper was developed for the diagnosis of *B. gibsoni* infection in dogs by PCR. This highly efficient DNA extraction method on DBS using Whatman 3MM filter paper has potential to be cost-effective and high-performance tool for storage, and molecular diagnosis of clinical blood sample from dog (Tani *et al.,* 2008). An improved elution protocol involving boiling of blood spots dried on Whatman filter paper was developed. DNA was efficiently extracted in phosphate-buffered saline (PBS) or Tris-EDTA (TE) buffer by incubation at 37°C overnight (Choi *et al.,* 2014).

Detergent proteinase K (DPK) method was developed for extracting *Theileria orientalis* DNA from bovine blood. This method gives reliable detection, genotyping, and quantification of this parasite relative to the widely used commercial kit (Bogema *et al*., 2015). The boiling method is almost restricted to the lysis step, and the subsequent purification and precipitation are not performed, making it less laborious. Nevertheless, it provides reasonable amounts of DNA that are usually enough to be used in amplification- based methodologies (Barbosa *et al*., 2016). Though there exist several DNA extraction methods, a simple and feasible method of DNA extraction for point of care diagnostics like LAMP is the need of the hour. Hence in this present study, simple methods of DNA extraction from blood were optimized and compared with kit method to assist LAMP.

MATERIAL AND METHODS

The DNA was extracted from the collected blood samples by four methods namely- Kit method, DPK method, boiling method, and Filter paper method. DNA yield was compared in each of the above-mentioned methods using UV spectrophotometer and LAMP.

Kit method: The DNA was extracted from the blood as per the manufacturer's protocol. Quantity of DNA was further assessed by UV spectrophotometer. The quality of genomic DNA extracted was determined by LAMP.

DPK method: The DNA was extracted as per the methodology of Bogema *et al*. (2015) with slight modification. Quantity of DNA was further assessed by UV spectrophotometer. The quality of genomic DNA extracted was determined by LAMP.

Boiling method: The DNA was extracted from the blood samples by boiling method described by Barbosa *et al.* (2016). Briefly, portions of whole blood are suspended in a lysis buffer containing a detergent, and a buffer solution. This suspension is incubated for 10–15 min at 95–100°C in a heat block or boiling water bath. Then, the tubes are briefly centrifuged to sediment the debris, and the supernatant is ready to be used. The quality of genomic DNA extracted was determined by LAMP.

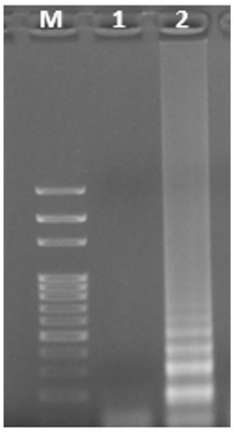
Filter paper method: DNA was extracted as per the methodology of Zhou *et al.* (2006). An aliquot of 200µl of 20mM NaOH solution was added to the tube containing the blood disk and it incubated for 30min at room temperature. The solution was then discarded, and the disk was washed in 200µl TE buffer for 2min. After the removal of the TE buffer, the disk was air-dried. The quality of genomic DNA extracted was determined by LAMP.

Statistical analysis: The DNA yield in both Qiagen kit method and DPK method was compared using t-test. And the data generated was analysed.

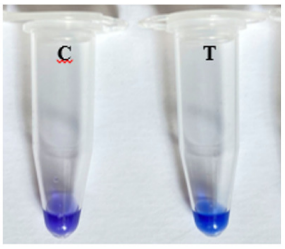
RESULTS AND DISCUSSION

The DNA yield in both Qiagen kit method and DPK method was compared using UV spectrophotometer. It was found that a higher yield was obtained with the DPK method when compared to Qiagen kit method. The average DNA yield in case of Qiagen kit method and DPK method was 13.84±1.22 ng/µl and 145.80±7.20 ng/µl respectively. On statistical analysis using t-test, the data sets were found to be highly significant between the groups. This indicates that there is a significant difference in the DNA yield between the two methods. The same results were indicated by Bogema *et al*. (2015). The reason could be due to the difference in collection technique. DNA collection is based on precipitation in the DPK method, and presumably allows for more thorough recovery. The kit method and DPK method both provided successful amplification with clearer bands on agarose gel. In colorimetric LAMP, the color changed from violet to blue (Fig. 1 and 2 respectively).

The DNA yield could not be evaluated using UV spectrophotometer in case of boiling method because of haeme interference. Hence, they were directly evaluated by LAMP for amplification. The DNA was extracted from *Theileria annulata* PCR positive blood samples and was directly used for LAMP reaction. Like any other DNA extraction protocol, the boiling method presents advantages and disadvantages. However, due to its low yield and purity, its usage in subsequent analytical procedures must be validated, since it may not be suitable for every downstream application (Queipo-Ortuño *et al*., 2008; Ahmed *et al*., 2014). Nevertheless, 10µl of the supernatant from the boiling method provided successful amplification with clearer bands on agarose gel. The major drawbacks are the low DNA yield and purity that are the result of the detergent usage, and the remaining cell debris that can contaminate the DNA samples and may inhibit the amplification methodologies. However, most amplification techniques are robust enough to be unaffected by inhibitors and require very low amounts of target DNA (Barbosa *et al.,* 2016). In colorimetric LAMP, the color changed from violet to magenta (Fig. 3). This alteration in the color change is due to the haeme interference in the assay. The DNA yield could not be evaluated using UV spectrophotometer in case of filter paper method because the DNA was bound to Whatman filter paper. Hence, they were directly evaluated by LAMP for amplification. The DNA was extracted from *Theileria annulata* PCR positive blood samples on the filter paper and the filter paper was directly used for LAMP reaction.

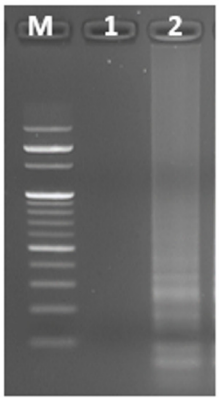


Lane M: 100 bp DNA ladder
Lane 1 : NTC (Non template control)
Lane 2 : Positive reaction indicating successful DNA extraction from kit method

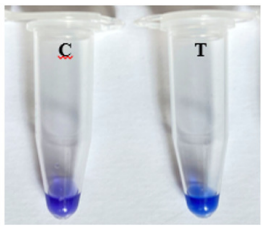


C: Negative reaction (Violet colour)
T: Positive reaction (Blue colour)

Fig. 1. LAMP using DNA extracted from kit method for detection of *Theileria annulata.*

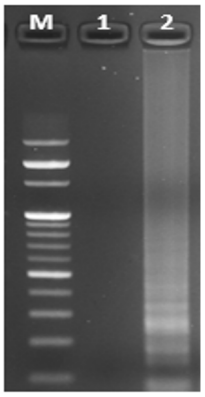


Lane M: 100 bp DNA ladder
Lane 1: NTC (Non template control)
Lane 2: Positive reaction indicating successful DNA extraction from DPK method

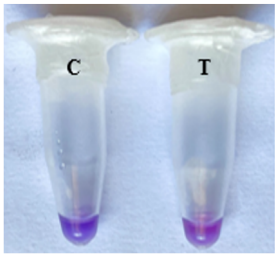


C: Negative reaction (Violet colour)
T: Positive reaction (Blue colour)

Fig. 2. LAMP using DNA extracted from DPK method for detection of *Theileria annulata.*

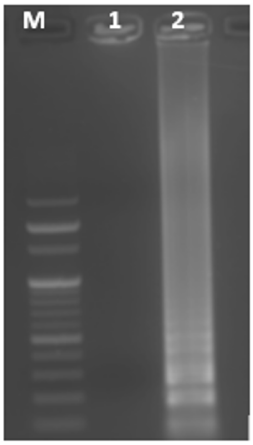


Lane M: 100 bp DNA ladder
Lane 1: NTC (Non template control)
Lane 2: Positive reaction indicating successful DNA extraction from boiling method



C: Negative reaction (Violet colour)
T: Positive reaction (Magenta colour)

Fig. 3. LAMP using DNA extracted from boiling method for detection of *Theileria annulata.*



Lane M: 100 bp DNA ladder
Lane 1: NTC (Non template control)
Lane 2: Positive reaction indicating successful DNA extraction from filter paper method

Fig. 4. LAMP using DNA extracted from paper method for detection of *Theileria annulata.*

And successful amplification was observed with this filter paper method (Fig. 4). But this method was found to be more useful in wet LAMP in comparison to colorimetric LAMP as it interfered with the color change. However, this method can be optimized for colorimetric LAMP using dyes other than HNB. The

use of dried blood spots on filter paper becomes an attractive alternative to blood collection in tubes for PCR analyses. First, only a small amount of blood is needed, and immediate processing is not required. Also, the sample transport and storage for dried blood spots is easy and less expensive. As a result of these advantages, Filter paper method has been widely used for the amplification assays (Zhou *et al.,* 2006).

CONCLUSIONS

As a result, a higher yield was obtained with the DPK method when compared to Qiagen kit method. Qiagen kit method is the most expensive of all the four methods, whereas DPK method is the most laborious and time consuming of all the four methods. Boiling method provides reasonable amounts of DNA that are usually enough to be used in LAMP. It is also very easy and inexpensive. The use of dried blood spots on filter paper becomes an attractive alternative to blood collection in tubes for LAMP analysis. Sample transport and storage for dried blood spots is easy and less expensive.

FUTURE SCOPE

These simple methods of DNA extraction would help in the downstream applications like Loop mediated isothermal amplification (LAMP). And LAMP could serve as a point of care diagnostics for many diseases of economic concern.

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Conflict of Interest. None.

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