# Short overview of optimised investigation procedure for the detection of waterborne parasites involving LAMP - loop mediated isothermal amplification


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*Giardia* and *Entamoeba* cysts and *Cryptosporidium* oocysts

## ABSTRACT


There is an ongoing demand for the development of different molecular tools with the aim of realising an efficient method that is simple, fast, and economic for the effective detection of life threatening microorganisms in water. In principle, the loop mediated isothermal amplification (LAMP) has been advocated as a unique, easy and low cost genetic analysis tool for resource-poor settings with increased sensitivity and specificity for the accurate detection of human pathogenic waterborne protozoan in the field or at the point of care by clinicians.

LAMP has been successfully applied for several taxa including *Cryptosporidium* spp., *Toxoplasma gondii*, *Giardia duodenalis, Entamoeba histolytica,* and matrices like stool, blood, tissues, and environmental samples. Here, we demonstrate the most significant waterborne parasites that are detectable using LAMP. A unified strategy showing the investigation procedure comprising of sample collection, purification, segmentation, and molecular analysis will be presented, as well as the advantages and disadvantages of LAMP assay in comparison to PCR assay.

The synergy of using this innovative and rapid assay indicates the important impact in the event of outbreaks caused by life threatening pathogens and for tracking the source of contamination. The combination of the LAMP together with the already existing PCR, and the sequencing and genotyping of the end product, provides valuable information to scientists and physicians for prevention, safety and the health of the population.

LAMP assay is valuable for medical outcomes (diagnoses), for tracking the source of infections and for monitoring purposes. Its economic importance is also significant for the detection of parasites at the earliest possible stage in the field.


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## INTRODUCTION

The emerging amplification method based on the specific detection of genomic DNA called loop-mediated isothermal amplification (LAMP) has not become common in Europe, yet.

Worldwide LAMP has been utilized for a broad spectrum of applications in the biomedical field inter alia the detection of viruses, bacteria, fungi, and pararasites[2].

### The LAMP principle

The LAMP method is a one-step amplification reaction amplifying a target DNA sequence with high specificity, sensitivity, high efficiency and rapidity under isothermal conditions using the *Bst* polymerase with strand displacement activity[14]. The mechanism of the LAMP reaction is consisting of three major steps, an initial step, a cycling amplification step, and an elongation step. The LAMP reaction employs the *Bst* polymerase, the reaction buffer, the DNA template and the primers, recognizing six or eight distinct regions within the target DNA (Fig. 1).

For the developement of the species specific LAMP method, we suggest the unified strategy in Fig. 2. After the evaluation, the LAMP could be applied in environmental samples following the strategy suggested in Fig. 3.

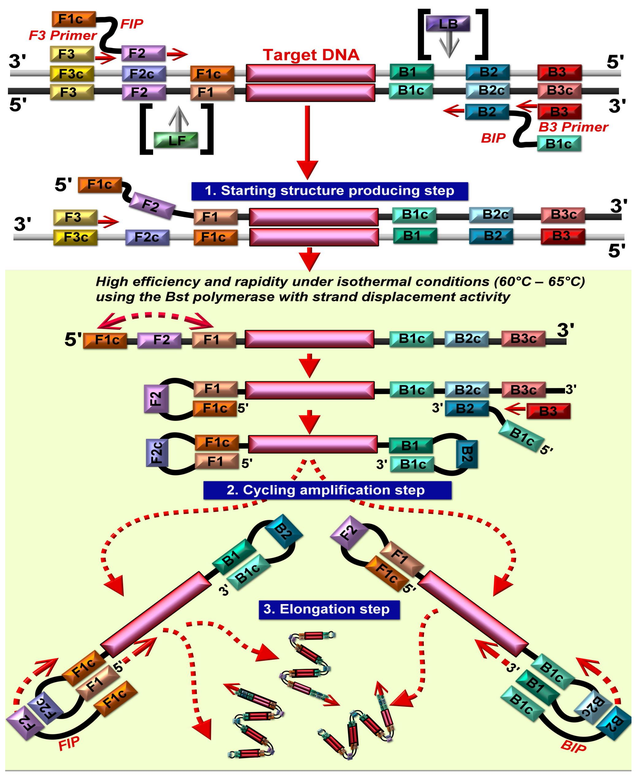


**Figure 1.** Simplified schematic representation of the major steps of the LAMP method and the localization of the 6 or 8 LAMP primers on the target DNA sequence for specific amplification.

## MATERIALS AND METHODS

This review aimed to assess whether the LAMP assay was developed and/or applied for the detection of waterborne protozoan parasites in the scientific field.

A literature search of PubMed, Medline and other electronic databases was conducted to obtain relevant publications, carried out up to July 2016. Two reviewers independently extracted data and assessed the methodological quality. In the electronic database the genera of fifteen protozoic parasites (Tab. 1) and the terms LAMP or loop mediated isothermal amplification were exerted into the search mask. The articles meeting the inclusion criteria, were considered as appropriate after critical review.

## RESULTS

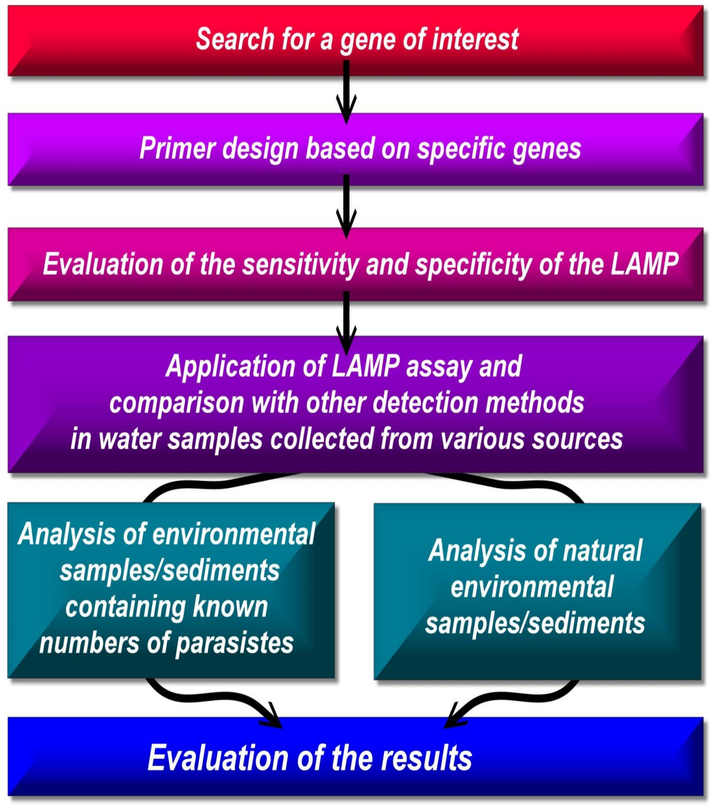


**Figure 2**. Schematic diagram demonstrating the unified strategy for the development of the LAMP.

| Term | Articles critically revised | Matrix |
|---|---|---|
| ***Acanthamoeba*** | **3** | Cornea, contact lens |
| ***Babesia*** | **8** | Blood, organs, horse, dogs, cattle, sheep, goat |
| ***Balantidium*** | **0** | |
| ***Blastocystis*** | **0** | |
| ***Cryptosporidium*** | **12** | **Water (8),** feaces |
| ***Cyclospora*** | **0** | |
| ***Entamoeba*** | **3** | Liver abscess, feaces |
| ***Giardia*** | **8** | **Water (4),** feaces |
| ***Isospora*** | **1** | Feaces |
| ***Microsporidia*** | **7** | Faeces, honeybees, silk worm eggs, shrimp, crayfish |
| ***Naegleria*** | **1** | Water |
| ***Sappinia*** | **0** | |
| ***Sarcocystis*** | **1** | Food (horse meat) |
| ***Toxoplasma*** | **26** | **Water (7),** soil, urin, blood, mice, cerebrospinal fluid, lymphnodes, pigs, CNS |
| **Total** | **70** | |
| **LAMP#** | **840*** | |
| **Loop mediated isothermal amplification#** | **1488*** | |
| **PCR#** | **393.793*** | |
| **Polymerase chain reaction#** | **495.120*** | |

**Table 1**. List of protozoic parasites (genus) and No. of research articles related to the detection by LAMP with special focus on water (*No. of articles listed in PubMed by searching the term#).

Out of seventy LAMP related articles, dealing with the detection of protozoic parasites, only sixteen articles were related to water; of these articles four included detection methods for two different species. In most of the published articles the LAMP method was evaluated or applied in matrices like blood, tissue, faeces, but not water. Out of the fifteen genera, the LAMP in water samples was successfully used for the detection of *Cryptosporidium*, *Giardia*, *Naegleria* and *Toxoplasma*[1, 3-13, 15-18] (Tab. 1).

The initial literature search (www.google.com) yielded 1.488 (0,3) publications for the term loop mediated isothermal amplification in comparison to 495.120 for polymerase chain reaction.

## DISCUSSION

Although LAMP has already been developed[14], it has not been applied on a wider scientific range yet, as the literature search demonstrated. On the other hand the call for surveillance systems and the investigation over suitable methods is arising, aiming to provide more sufficient results concerning the infections caused by the parasitic organisms.

We have already developed and applied the LAMP method for the detection of the apicomplexan *Cryptosporidium* species, *Toxoplasma gondii* and the flagellata *Giardia duodenalis,* other scientists approved for further waterborne protozoa that LAMP is sensitive and specific. All in all, LAMP is much more easy and cost effective than PCR.

### Sample analysis strategy




**Figure 3**. Schematic diagram demonstrating the unified detection strategy (sampling, preparation and LAMP assay).

## CONCLUSION

Water is worth protecting and the most important foodstuff and its contamination by parasites is a health risk affecting all of us. Infective stages of protozoan parasites are able to persist in the aquatic environment for months and the water is the main route of infection. We want to advocate the LAMP method as an economic assay to detect waterborne parasites by fastest possible processing in cases of outbreaks.

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