DEVELOPMENT AND TESTING OF LAMP ASSAY FOR DIAGNOSIS OF *PLASMODIUM FALCIPARUM* MALARIA

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Malaria is one of the three major infectious diseases in the world. The malarial parasite, *Plasmodium falciparum*, causes an estimated two million deaths each year, primarily in children under the age of five years. Although the parasite has become resistant to some drugs, individuals can still be treated and cured if the disease is diagnosed early and accurately. The objective of this study was to develop a simple and rapid diagnostic test that can be used in malaria-endemic areas. Microscopic detection of the malarial parasites in human blood smears has long been used as a gold-standard for clinical diagnosis. Alternatively, nested polymerase chain reaction (PCR) has been used because of its higher sensitivity. Nevertheless, microscopy and nested PCR require extensive training, high-end resources and time.

Recently, the loop-mediated isothermal amplification (LAMP) method has been developed to detect similar microbial infections. In my study, the LAMP assay was used to detect *P. falciparum* and the results were compared with traditional microscopy and nested PCR. A total of 25 blood samples from Cameroon and Thailand were obtained from consenting adults. Based on microscopy, 10 samples were negative and 15 were positive for *P. falciparum*. These 25 samples were coded and analyzed using nested PCR with purified DNA and using LAMP with both purified DNA and whole blood. The ultimate goal of this study was to evaluate the utility of the LAMP assay for diagnosis of malaria in developing countries.

BACKGROUND

Current methods for detection of *P. falciparum* contain unique benefits, yet still face their obstacle. PCR amplifies low levels of DNA to higher readable amounts. Though highly accurate, the time and resources required inhibit it from being used in clinical settings. Microscopy can and is used in the clinical setting, but again, has too many requirements. First, there's the need for electricity to power light microscopes; second, many laboratories in developing countries are too poor to buy microscopes; and third, a person with extensive training is needed to identify parasites in a smear of blood. Rapid chromatographic tests have also become available as an indicator of the *P. falciparum* parasite by detecting malarial proteins in blood. While it is a fairly simple method of detection, it lacks the sensitivity required to diagnose patients with low parasitemia. Thus, the need for a simple, accurate and rapid method of diagnosis of *P. falciparum* is essential. LAMP holds great potential to become the new standard for diagnosing malaria.[1–2] This assay has been used to diagnosis other infectious diseases, such as the hepatitis B virus.[3] LAMP has the ability to amplify DNA into mass quantities at an isothermal temperature between 60–65°C, for a mere 60–100 minutes and fluorescence detection using Syber Safe I, make it an ideal assay.

METHODS

DNA was extracted from 25 samples to be tested. With the purified DNA, nested PCR was conducted. The gene that codes for ribosome in *P. falciparum* species was detected during the first round of amplifications, and then the product was amplified using primers specific for the ribosome gene of *P. falciparum*. The thermocycler would run through 25–30 cycles (*Plasmodium* species and speciation, respectively) of annealing, extension and denaturation, to amplify the targeted DNA. The final product was then run through a 2% agarose gel electrophoresis to read out the results. Expected bands for *P. falciparum* are located at 205 bp.

The same 25 DNA samples and whole blood are used for the LAMP assay. By combining 3 sets of primers, along with other reagents, and either purified DNA or heat denatured blood (at 95°C), the samples were submerged into a water bath. In the bath, the sample incubated between 60–65°C for approximately 60 minutes. After the amplification process, the samples are put into another water bath set to 80°C to inactivate the enzymes. Syber Safe I is then added for fluorescence detection under ultra violet light.

RESULTS

After PCR amplification and running the gel, all 10 negative samples matched the microscopy results. Thirteen of the 15 positive samples matched the results based on microscopy; when the 2 false negative samples were run through PCR a second time, the parasites were detected. PCR was 100% compared to microscopy. The LAMP assay however was not as

synchronized. LAMP consistently produced unspecific amplification, resulting in continuous false positives. Numerous attempts were made to optimize the assay; all working stock were changed to eliminate the possibility of contamination, concentrations of magnesium, Bst Polymerase and primers were altered (all known to have an effect on DNA amplification). Different temperatures and durations of incubation were tested; none of which had any affect.

CONCLUSION

From this study, PCR continues to be highly sensitive and accurate; but it cannot be used in developing countries because of the resources required. Unfortunately, LAMP also cannot be transferred due to its unspecific amplification. It does hold high potential; it is quick, easy and precise. With continued efforts in the optimization of this assay, perhaps it may eventually become a new standard for the diagnosis of *P. falciparum*.

REFERENCES

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