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学位の種類	博士（医学）
学位記番号	博甲第　９２４３　号
学位授与年月	令和元年５月３１日
学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Performance of Malaria Diagnosis by a PURE-LAMP System and its Application to Screening for Antimalarial Drug Resistance Molecular Surveillance （PURE-LAMP システムのマラリア 診断法としての有用性及びその薬剤耐性マラリア分布調査への応用）

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論文の内容の要旨
Abstract of thesis

In this dissertation, Jeanne Perpetue Vincent describes on the performance of malaria diagnosis with the PURE-LAMP system in a laboratory setting and the application to the screening of antimalarial drug resistance surveillance in a field setting in Haiti. The content is summarized as follows:

（目的 Purpose）

Malaria, one of the most important parasitic infectious diseases for which almost half of the world's population is at risk is caused by *Plasmodium spp*. There is still a need of highly sensitive diagnostics for its detection. The loop-mediated isothermal amplification (LAMP) method is a DNA amplification tool in which the DNA amplification can be achieved by incubation at a stable temperature. A malaria detection kit based on this methodology has shown promising results in previous studies. In this thesis, the author aimed at evaluating this method as a part of field-friendly system, using DNA extracted from dried blood spots by a simple DNA extraction method, the procedure for ultra-rapid extraction (PURE), which can produce a DNA solution suitable for the

LAMP reaction without the use of a centrifuge. Hypothesizing that this method is more sensitive than microscopy, another objective was to estimate the proportion of antimalarial resistant isolates that are found among submicroscopic infections detected by PURE-LAMP.

(対象と方法 Materials and Methods)

Blood samples were tested by a rapid diagnostic test (BinaxNow® Malaria, Alere Inc., USA), microscopy, PURE-LAMP and nested PCR. The sensitivity and specificity of each method were estimated with nested PCR as a gold standard. The analysis was conducted retrospectively with samples collected from 2011 to 2016 at the National Center for Global Health and Medicine in Tokyo.

The PURE-LAMP was subsequently applied as a screening method in a malaria endemic country, Haiti. Positive samples by the PURE-LAMP were targeted for genotyping of resistance markers for two antimalarial drugs: chloroquine and artemisinin. The PURE-LAMP as a screening method was further compared with the results of microscopy and rapid diagnostic test.

(結果 Results)

The author tested a total of 117 samples including 46 *Plasmodium falciparum*, 7 *P. vivax*, 9 *P. ovale*, 4 *P. malariae*, and 51 negative cases. The PURE–LAMP Pan (targeting *Plasmodium* genus) correctly identified 64 of the 66 positives and the 51 negatives. Among the Pan-positive samples 45 *P. falciparum* were also detected with the PURE–LAMP Pf (targeting *P. falciparum* specifically*)*. The PURE–LAMP Pan and PURE–LAMP Pf had respective sensitivities of 96.96% (95% CI 89.47–99.63) and 97.82% (95% CI 88.47–99.94) and common specificity of 100%.

During the field survey, among 98 samples detected positive by the PURE-LAMP Pf, 89 samples were successfully sequenced for *P. falciparum* chloroquine resistance transporter, a chloroquine resistance marker and 85 samples were successfully sequenced for *kelch 13*, an artemisinin resistance marker. Rapid diagnostic test and microscopy detected 76 and 44 respectively of those analyzed positive samples. All samples presented sensitive genotypes.

(考察 Discussion)

Obtained sensitivity and specificity by the retrospective study were as expected based on previous publications, showing that the Loopamp kit still performs well with DNA extracted by the PURE method from dried blood spots. In malaria endemic field it allows detection of infections (symptomatic or not) missed by light microscopy or rapid diagnostic test. The study could not estimate the proportion of resistant genotypes that came from submicroscopic infections because all the sequenced samples presented sensitive genotypes.

No drug resistance-associated mutation was detected in *P. falciparum* chloroquine resistance transporter nor in *kelch 13* of the isolates from Haiti. This confirms chloroquine as the drug of choice for treatment of uncomplicated malaria in Haiti, unlike the global policy for treatment of *P. falciparum* infection, and artemisinin as a valid alternative. The contribution of submicroscopic

infections to the resistant parasites pool is needed to be studied elsewhere.

(結論 Conclusion)

Malaria detection by PCR is only available at the National Laboratory of Public Health in endemic countries. The PURE-LAMP would have the effect of decentralizing nucleic acid amplification techniques for detection of parasites, bringing it to a more regular practice. Subsequently more samples would have been analyzed by nucleic acid amplification techniques and, thus low parasitemic infections can be found and treated. That would help clearing the infectious reservoir and facilitate the elimination of malaria faster.

審査の要旨
Abstract of assessment result

【批評 General Comments】

Global disease control strategies of malaria are currently moving towards its elimination. On this platform, we require further sensitive and appropriate technique to detect low parasitemic infections. The author tested a PURE-LAMP system for its reliability using archived samples in the laboratory, then moved to examine the usefulness in an endemic country setting. This technique has been developed recently and there is scarce information for its usefulness in endemic country settings. This study clearly demonstrated that the PURE-LAMP system has an advantage of simple procedure steps, and that if this new technique can be applied widely, then malaria elimination would be facilitated.

【最終試験の結果 Assessment Result】

The final examination committee conducted a meeting as a final examination on March 11, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

Therefore, the final examination committee approved that the applicant is qualified to be awarded the Doctor of Philosophy in Medical Sciences.