論　文　概　要

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

指導教員: 狩野繁之

筑波大学（連携）大学院 人間総合科学研究科 生命システム医学専攻　教授

筑波大学大学院人間総合科学研究科生命システム医学専攻

JEANNE PERPETUE VINCENT

Background：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 in endemic field. 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. This thesis aimed at evaluating this method as part of a 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.

Methods：Blood samples were tested by a rapid diagnostic test (BinaxNow® Malaria, Alere Inc., Waltham, MA, USA), microscopy, PURE-LAMP and nested PCR. The sensitivity and specificity of each method were estimated with nested PCR as 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. PURE-LAMP positive samples were targeted for genotyping of resistance markers for 2 antimalarial drugs: chloroquine and artemisinin. PURE-LAMP as a screening method was further compared to microscopy and rapid diagnostic test (SD Bioline Malaria Ag Pf/ Pan; Standard Diagnostics, Inc., Suwon, South Korea).

Results：One hundred and seventeen samples including 46 *Plasmodium falciparum*, 7 *P. vivax*, 9 *P. ovale*, 4 *P. malariae*, and 51 negative cases were tested. 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 in Haiti, among 98 samples detected positive by 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 phenotypes.

Discussion and Conclusion: The 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 the endemic field, it allowed detection of infections (symptomatic or not) missed by light microscopy or rapid diagnostic test. Thus, this system (PURE-LAMP malaria on dried blood spots) is a valuable tool for malaria mass screening.

The proportions of resistant genotypes from microscopic or submicroscopic infections could not be estimated as all sequenced samples presented the sensitive genotype. 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 trend for treatment of *P. falciparum* infection, and artemisinin as a valid alternative. The contribution of submicroscopic infections to the resistant parasites pool is still needed to be studied elsewhere.