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Evaluation of Non-Instrumented Nucleic acid Amplification- Loop Mediated Isothermal Amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia

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Background: Malaria is the major public health problem in sub-Saharan African countries including Ethiopia. Early and accurate diagnosis followed by prompt and effective treatment is the main strategy for prevention, control and elimination of malaria. Hence, highly sensitive, specific and rapid molecular methods are urgently needed for diagnosing malaria and differentiating *Plasmodium* spp. parasite infections. However, high cost and infrastructure limitations are significant hurdles to the introduction of molecular diagnostics in low-resource settings in endemic areas. Therefore, this study aimed to evaluate the performance of non-instrumented nucleic acid amplification- loop-mediated isothermal amplification (NINA-LAMP) compared to standard thick and thin films and nested PCR as gold standard for the diagnosis of malaria in Northwest Ethiopia.

Methods: A cross sectional study was conducted in North Gondar, Ethiopia from March to July 2014. Eighty two blood samples were collected from malaria suspected patients visiting Kola Diba Health Center and analyzed for *Plasmodium* parasites by Giemsa microscopy, NINA-LAMP and nested PCR. The LAMP method was performed using the LoopampTM Malaria Pan/Pf detection kits (Eiken Chemical, Japan) and a NINA heater (PATH, Seattle, WA) for detecting DNA of the genus *Plasmodium* and more specifically *Plasmodium falciparum*. Data were analyzed by SPSS version 20 and MedCalc online software. Diagnostic accuracy outcome measures (sensitivity, specificity, and predictive value and Kappa value) of NINA-LAMP and Giemsa microscopy were compared to nested PCR as reference method.

Results: A total of 82 samples were tested in the primary analysis. Using nested PCR as a reference, the sensitivity and specificity of the primary NINA-LAMP assay were 96.77% and 84.31%, respectively for detection of *Plasmodium* genus, and 100% and 81.16%, respectively for detection of *P. falciparum* parasite. Microscopy demonstrated sensitivity and specificity of 93.55%, 98.04%, respectively for the detection of *Plasmodium* parasites. Post hoc repeat NINA-LAMP analysis showed significantly improved diagnostic accuracy which was comparable to nested PCR performance and superior to microscopy for detection at both the *Plasmodium* genus level and *P. falciparum* parasites.

Conclusion: In our study, NINA-LAMP is highly sensitive and specific for the diagnosis of malaria and detection of *Plasmodium* parasite infection at both the genus and species level when compared to nested PCR. NINA-LAMP is likely more sensitive for the detection and differentiation of *P. falciparum* from non-falciparum species compared to Giemsa microscopy and may be a critical diagnostic modality in efforts to eradicate malaria from areas of low endemicity.

Biography

Meslo Sema completed his graduation from Addis Ababa University, Ethiopia with very great distniction remark. He also completed his MSc in Medical Parasitology at the age of 24 years from University of Gondar, Ethiopia. He was doing his MSc thesis on malaria new innvovative molecular diagnostic evaluation for the first time by using LoopampTM Malaria Pan/Pf detection kits (Eiken Chemical, Japan) and a NINA heater (PATH, Seattle, WA). Now he is working as lecturer and researcher at college of Medicine and Health science, Wollo University, Ethiopia.

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