NOVEL ISOTHERMAL NUCLEIC ACID AMPLIFICATION METHOD FOR DETECTION OF *PLASMODIUM* SPECIES: VALIDATION IN BRAZILIAN-MALARIA ENDEMIC AREAS

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INTRODUCTION: Malaria is a parasitic disease caused by *Plasmodium* spp. and transmitted by anopheline mosquitoes. Despite being endemic in tropical and subtropical regions, malaria remains a major global health issue, with an estimated 249 million cases and 608,000 deaths in 2022. Rapid and accurate diagnosis and treatment are crucial for malaria control and elimination. However, limited access to sensitive molecular tests means that microscopic examination and Rapid Diagnostic Tests (RDT) are the most used methods in endemic areas, despite their lower diagnostic accuracy. Therefore, there is a need for developing sensitive, simple, accurate, and rapid diagnostic tools suitable for field conditions. **OBJECTIVES:** Herein, we aimed to explore the potential of the enzymatic recombinase amplification assay (ERA® Technology) as a remote laboratory test by evaluating and validating the GENEYE® ERA *Plasmodium* detection kit in Brazilian endemic areas. **METHODOLOGY:** A cross-sectional cohort study was conducted between June and August of 2023 in the Brazilian Amazon. The study enrolled 323 participants residing in three malaria-affected regions: Cruzeiro do Sul and Mâncio Lima (Acre State) and Guajará (Amazonas State). The participants were tested for malaria by microscopy, RDT, nested PCR (nPCR), real-time PCR (qPCR) and ERA. The sensitivity, specificity, and predictive values were assessed using nPCR as a gold standard. **RESULTS:** *Plasmodium* prevalence was 21,7%, 18,8%, 19,2%, 21,7%, and 21,7% by nPCR, microscopy, RDT, qPCR, and ERA respectively. Using nPCR as the standard, qPCR, and ERA showed a sensitivity of 100%. In comparison, microscopy and RDT showed a sensitivity of 87,1% and 88,6%, Negative Predictive Value (NPV) of 96,56 and 96,93, and *Kappa* values of 0,91 and 0,92, respectively. For *P. falciparum*, the sensitivity of qPCR and ERA was 100% while the sensitivity of microscopy and RDT was 96,9% and 93,7%, and the NPV was 99,66 and 99,32, respectively. For *P. vivax*, only ERA showed the same sensitivity of nPCR. The sensitivity, NPV, and *kappa* values were 78,85%, 97,27 and 0,87 for qPCR and microscopy, and 84,21%, 97,94 and 0,9 for RDT. **CONCLUSION:** The data demonstrate that the GENEYE® ERA *Plasmodium* detection kit is a promising alternative to traditional malaria diagnostic methods. Its high sensitivity, specificity, fast processing, and simplicity makes it a valuable point-of-care diagnostic tool, particularly in resource-limited and remote malaria-endemic areas.

KEYWORDS: *Plasmodium* Detection; Isothermal Nucleic Acid Amplification; Rapid Diagnostic Tests; Point-of-Care; Brazilian Amazon; Malaria.

FUNDING: This work was supported by FIOCRUZ (INOVA - VPPCB-007-FIO-18-2-46), CNPq (425.422/2016-0), PRONEX, FAPERJ (E-26/010.001268/2016) and CAPES (Finance Code 001).