IVD_21 - Development and proof of concept of a multiplex molecular assay for Plasmodium species screening by real time PCR

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Introduction: Malaria disease, caused by a protozoa genus *Plasmodium*, is a parasite infection spread to humans by the bites of female mosquito genus *Anopheles*. It has prevalence around the tropical zone area mostly in Africa and South America. Although, considering autochthonous cases, Malaria has been carried out as global importance for epidemiological and surveillance studies. The Brazilian Amazonia Forest is one of the most critical areas for Malaria screening, due the frequence of cases. The Instituto Evandro chagas, as reference laboratory for Malaria disease, and Bio-Manguinhos in a partnership, comes with a molecular assay for ultra- sensitive Malaria detection and screening of species by a real time PCR multiplex.

Objectives: The main purpose of this study is to present the proof of concept of the assay for Malaria detection using reference samples.

Methodology: Detecting 5 targets, the assay can distinguish *Plasmodium falciparum* (Pf), *Plasmodium vivax* (Pv) and *Plasmodium malariae* (Pm) with high specificity. Also, we included a target for Pan-*Plasmodium spp* (PanP) detection using an inter-species conservative region at the *RNA 18S* gene. As an internal control, human RNAse P gene was selected. The dyes and cycling conditions were selected and optimized previously using reference samples.

Results: The proof of concept was carried out testing 140 blood samples, including 3 types: blood spot on paper (84), scraped thick blood drop on slide (24) and total blood (31). As the results, the total blood samples were in concordance with reference previous results. For paper, 43 samples were negative and 41 positive – 4 Pf, 34 Pv and 1 positive both targets. Also, 2 samples were positive only in the PanP. Using extracted blood from slide, 21 Pf positive samples were found, 2 Pv and 1 negative with more than 92% concordance.

Conclusion: As a conclusion, the proof of concept had carried out showing important results which confirmed the efficiency, specificity, and robustness of the assay under development. As conclusion, it was evaluating the design and first steps of the standardization. With this, the agreement of the results allows us to keep going and test other parameters, like different sample extraction methods increasing sensitivity for detection.

Keywords: Malaria detection; Plasmodium species