Genomic investigation of transfusion-transmitted malaria in Brazil: Development of a multiplex molecular assay and its application to hemovigilance

Pedro H. Cardoso[1,2]; Mônica B. Arruda[2]; Patrícia A. Baptista[2]; Claudio Tadeu Daniel-Ribeiro & Maria de Fátima Ferreira-da-Cruz[1]

[1]Laboratório de Pesquisa em Malária, IOC/FIOCRUZ-RJ; Centro de Referência SVSA/MS
[2]Laboratório de kits Moleculares, Bio-Manguinhos, FIOCRUZ-RJ

Introduction: Transfusion-transmitted malaria remains a persistent risk in low-endemicity settings, particularly because of asymptomatic donors with submicroscopic parasitemia. In Brazil, NAT Plus-based molecular screening at blood centers has created a strategic opportunity to integrate sensitive diagnosis and genomic surveillance. This approach aligns transfusion safety with national malaria elimination goals. **Objectives:** To develop a discriminatory multiplex molecular assay capable of detecting *Plasmodium vivax/P. simium* (Pv/Ps), *P. falciparum* (Pf), and *P. malariae/P. brasilianum* (Pm/Pb) with potential application in the screening and genomic characterization of NAT Plus-positive samples from the Brazilian blood network. **Methods:** SSU 18S rRNA genomic regions for species differentiation were analyzed, enabling construction of a multiplex real-time PCR assay that detects PAN-*Plasmodium*, Pv/Ps, Pf, Pm/Pb, and a human internal control (RNase P). Seventy-seven blood samples detected as malaria-positive by the NAT Plus HIV/HBV/HCV/Malaria kit (Bio-Manguinhos) were selected for analytical typing and *Plasmodium* evaluation by real-time PCR and confirmation by high-throughput sequencing (HTS) on the Nanopore platform (MinION). **Results:** *In silico* analysis identified regions with an optimal balance between conservation and interspecies discriminatory power. Overall, 24 samples were Pm/Pb positive, 46 for Pv/Ps, and 7 for Pf. No co-infection was observed. Ten samples were selected for species confirmation, indicating 100% concordance between molecular detection and genomic typing, reinforcing the assay's feasibility for hemovigilance applications. **Conclusion:** The multiplex assay demonstrates the potential to expand detection of submicroscopic *Plasmodium* infections in blood donors by integrating sensitive diagnosis with applied genomics. A remaining challenge is identifying markers to characterize *P. malariae* diversity, given its impact on blood donor eligibility.

Keywords: transfusion-transmitted malaria; hemovigilance; real-time PCR; genomics; *Plasmodium*.