A rapid immunochromatographic test for antibody detection as a new tool for monitoring asymptomatic infection with *Plasmodium vivax*

Julia R Sampaio[1], Fabiana F Coelho[1], Irene S Soares[2], Jéssica Karoline A Oliveira[1], Ana Clara G Guimarães[1], Alex F Carvalho[1], Bruno Vinicius S Valiate[1], Camila M Costa[3], Caroline F Junqueira[1], Alexia M V Silva[4], Michelle Oliveira-Silva[4], Dhelio B Pereira[4], Anielle de Pina-Costa[5,6], André M Siqueira[6], Márcia C Castro[7], Renan P de Souza[1], Lis R V Antonelli[3], Ana Paula S Fernandes[1*], Ricardo T Gazzinelli[1*], Gregório Guilherme Almeida[1,3*]

[1]Vaccine Technology Center (CT Vacinas), Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.
[2]School of Pharmaceutical Sciences, University of São Paulo (USP), São Paulo, SP, Brazil.
[3]René Rachou Research Institute, Oswaldo Cruz Foundation, FIOCRUZ-MG, Belo Horizonte, MG, Brazil.
[4]Tropical Medicine Research Center, Porto Velho, RO, Brazil.
[5]Fluminense Federal University (UFF), Niterói, Rio de Janeiro, RJ. Brazil
[6]Evandro Chagas National Institute of Infectious Diseases – INI, Oswaldo Cruz Foundation, FIOCRUZ, Rio de Janeiro, RJ, Brazil.
[7]Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, United States of America.

APSF, RTG, and GGA equally contributed to this study.

Introduction: Eliminating *Plasmodium vivax* malaria is challenging in low-transmission areas due to asymptomatic and subpatent infections that reduce the effectiveness of conventional diagnostics. Since the acquisition and maintenance of antimalarial antibodies depend on exposure to infection, antibodies can serve as biomarkers of transmission intensity, representing a promising alternative for monitoring recent exposure to the parasite and identifying individuals with low parasitemia or carriers of hypnozoites.

Objective: To develop and validate a rapid immunochromatographic test for the detection of IgG antibodies from asymptomatic *P. vivax* malaria.

Methods: The antigens were conjugated to colloidal gold nanoparticles, and anti-human IgG was immobilized on the nitrocellulose membrane as the test line. Test performance was evaluated using plasma from symptomatic and asymptomatic individuals, as well as from healthy donors from a non-endemic area. Field applicability was assessed in Rondônia, Brazil, using samples from treated symptomatic individuals and asymptomatic individuals followed over time.

Results: Under laboratory conditions, the prototype showed 93.7% sensitivity and 100% specificity. We then observed a gradual decrease in antigen-specific IgG responses after treatment, suggesting that positivity reflects recent exposure to the parasite or persistent infection. In longitudinal studies of asymptomatic individuals, positivity in the IgG rapid test and ELISA was strongly associated with the risk of subsequent detection of

parasitemia by qPCR, highlighting the test's usefulness in identifying individuals who were recently exposed and are more likely to be actively infected. Overall, under field conditions, the IgG rapid test showed a sensitivity of 80.10% and a specificity of 89.44%. **Conclusion:** The developed IgG rapid test is a promising tool for supporting *P. vivax* surveillance, particularly in monitoring transmission in areas moving towards eradication.