

Development of a Rapid Diagnostic Test for Malaria Antigens Detection using Gold Nanoparticles-Antibody Conjugates

Inês Gomes^{1,2}, Cláudia S. Cunha¹, Eulália Pereira³, Diane W. Taylor⁴, Maria M. Mota¹, Miguel Prudêncio¹, Ricardo Franco²

¹Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, 1649-028 Lisboa, Portugal.

²REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

³REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal.

⁴Department of Tropical Medicine, University of Hawaii, Honolulu, Hawaii 96813, U.S.A.

ines.gomes@fct.unl.pt

Our main objective is to design a gold nanoparticle (AuNP)-based rapid diagnostic test (RDT) using monoclonal antibodies to detect *P.falciparum*-specific antigens in clinical samples.

AuNPs are ideal candidates for biosensing assays due to their unique nanoscale properties, such as high surface areas, robustness, facile synthesis and functionalization, and strong, non-bleaching, optical absorptions [1, 2]. These properties are especially interesting for the development of colorimetric molecular assays that are inexpensive, easy to implement and interpret, and can be used in the form of a test-kit [3].

Our assay set intends to go beyond the state-of-the-art in commercially available RDTs, with increased sensitivity and stability imparted by optimized gold nanoparticles.

Gold nanoparticles are functionalized with mercaptoundecanoic acid (MUA), and conjugated with a monoclonal antibody (2G12) that specifically recognizes *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2), a malaria antigen frequently used in RDTs.

Conjugation of the monoclonal antibody with the functionalized AuNP was performed by electrostatic interactions or by covalent attachment, through the available antibody amine groups, allowing the formation of active and robust AuNP-antibody conjugates.

The formation, robustness and binding properties of the AuNP-MUA-2G12 conjugates was evaluated by agarose gel electrophoresis (Figure 1) and zeta potential measurements.

A Western blot analysis was performed to confirm if PfHRP2 can still be recognized by the monoclonal antibody when conjugated with AuNP-MUA.

We have recently started to develop a simple test on a nitrocellulose strip, based on a competitive format. PfHRP2 antigen will be coated on the test zone of the dipstick, capturing the AuNP-antibody conjugate and allowing the red color to concentrate and form a spot (Figure 2).

The immunoassay will be used for detection of a recombinant purified antigen and of the same antigen in malaria-infected *in vitro* blood cultures. This study will constitute an important proof-of-concept for future tests in clinical samples.

References

- [1] Baptista, P.; Doria, G.; Quaresma P.; Cavadas M.; Neves, C. S.; Gomes, I.; Eaton, P.; Pereira, E.; Franco, R. "Nanoparticles in Molecular Diagnostics" Progress in Molecular Biology and Translational Science: Nanoparticles in Translational Science and Medicine, Elsevier, 2011, 427-488.
- [2] Baptista, P.; Pereira, E.; Eaton, P.; Miranda, A.; Gomes, I.; Quaresma, P.; Franco, R. Anal Bioanal Chem, **391**(3), 2008, 943-950.
- [3] Azzazy, H. M. E.; Mansour, M. M. H.; Samir, T. M.; Franco, R. Clinical Chemistry and Laboratory Medicine, **50**(2), 2012, 193-209.

Figures

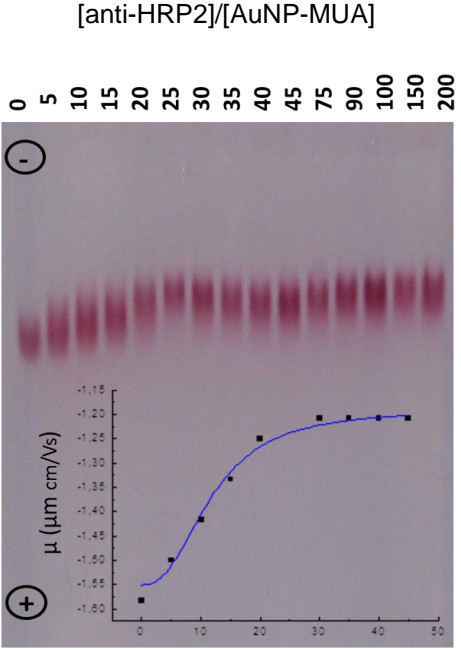


Figure 1 - Agarose gel electrophoresis of AuNP-MUA-anti-HRP2 conjugates. **Inset:** Electrophoretic mobility of the conjugates.

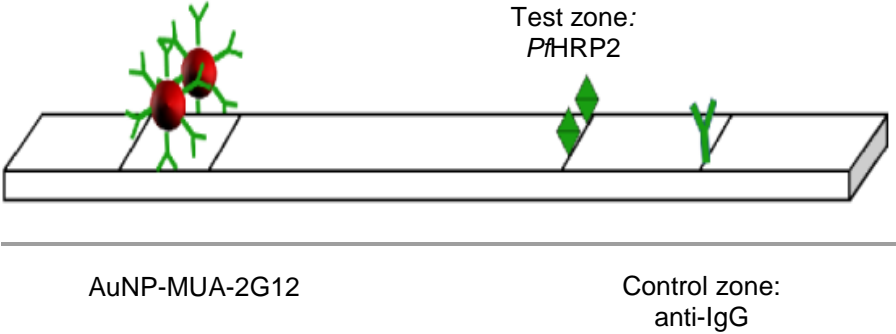


Figure 2 - Schematic representation of the Rapid Diagnostic Test.