# Bionanotechnology for Malaria Diagnostics: Towards a Point-Of-Need Assay

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## Abstract

Despite the fact that several countries were able to eradicate malaria during the past century, it remains one of the most prevalent infectious diseases worldwide. Forty percent of the world's population is at risk of infection, and 500 million people become infected every year [1]. Hopes for the eradication of this disease during the 20$^{th}$ century were dashed by the ability of *Plasmodium falciparum* (*Pf*), its most deadly causative agent, to develop resistance to available drugs. Despite its huge burden, the diagnosis of malaria is often not straightforward. If malaria rapid detection tests (RDT) reliably equal or surpass the efficacy of clinical microscopy, the accepted 'gold standard' despite its significant limitations, they could have a significant role in clinical practice [2].

We aim to design a gold nanoparticle (AuNP)-based rapid detection test (RDT) using specific antibodies to detect *Plasmodium falciparum* (malaria parasite) antigens in clinical specimens. The characteristics of the proposed malaria RDTs include reproducibility, acceptable high sensitivity and specificity, rapidity, ease of performance and interpretation, stability when stored, and capability of species differentiation, all at an affordable price.

We recently established the proof-of-concept for a competitive immunofluorescent assay using a *Plasmodium falciparum* heat shock protein 70 (*Pf*Hsp70) antigen/monoclonal antibody pair [3]. This homogeneous assay is based on the fluorescence quenching of cyanine 3B (Cy3B)-labeled recombinant *Pf*Hsp70 upon binding to AuNPs functionalized with an anti-*Pf*Hsp70 monoclonal antibody (Figure 1). Upon competition with the free antigen, the Cy3B-labeled recombinant *Pf*Hsp70 is released to solution resulting in an increase of fluorescence intensity (Figure 2). The estimated LOD for the assay is 2.4 $\mu g.mL^{-1}$ and the LOQ is 7.3 $\mu g.mL^{-1}$. The fluorescence immunoassay was successfully applied to the detection of antigen in malaria-infected human blood cultures at a 3% parasitemia level, and is assumed to detect parasite densities as low as 1,000 parasites.$\mu L^{-1}$.

*Pf*Hsp70 is nevertheless not the ideal antigen for malaria detection as a protein-sequence similarity search reveals 96-93% identity to other *Plasmodium* species, and some 80% identity to Coccidian and Cryptosporidiosis parasites. This result indicates that *Pf*Hsp70 monoclonal antibody might be unable to distinguish between *Plasmodium* species, and false positives could be potentially generated by the presence of other prevalent parasitic infections.

*Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP-2) is a water-soluble protein released from parasitized erythrocytes into *in vitro* culture supernatants, that has proven to be of interest for its potential effects on the host immune system and as an antigen for specific diagnosis of malaria [4]. We are using a *Pf*HRP-2 antigen/monoclonal antibody pair in the above fluorescent competitive immunoassay format hoping to confirm the validity of the assay and its specificity and sensitivity parameters also for this antigen/monoclonal antibody system. As a drawback for the utilization of this antigen in diagnosis, it has been found that detectable antigen frequently persists after parasite clearance. The search for new antigens for malaria diagnostics continues, with the aim of revealing more sensitive and disease-specific targets [5].

## References

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**Figures**

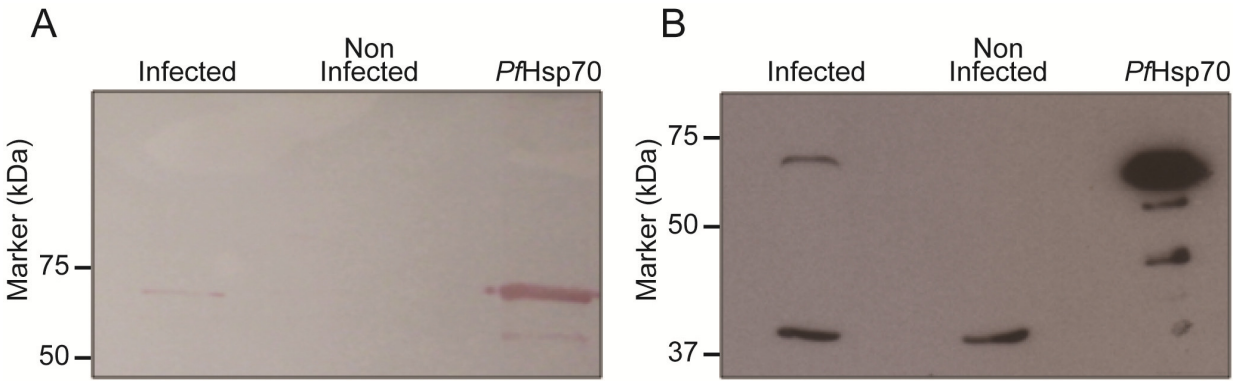


**Figure 1** - *Pf*Hsp70 detection on saponin-treated pellets of *P. falciparum*-infected RBCs of a human blood culture and of RBCs of non-infected human blood. (A) *Pf*Hsp70 detection (red bands) following incubation with AuNP-antibody conjugates. (B) *Pf*Hsp70 detection by chemiluminescence.

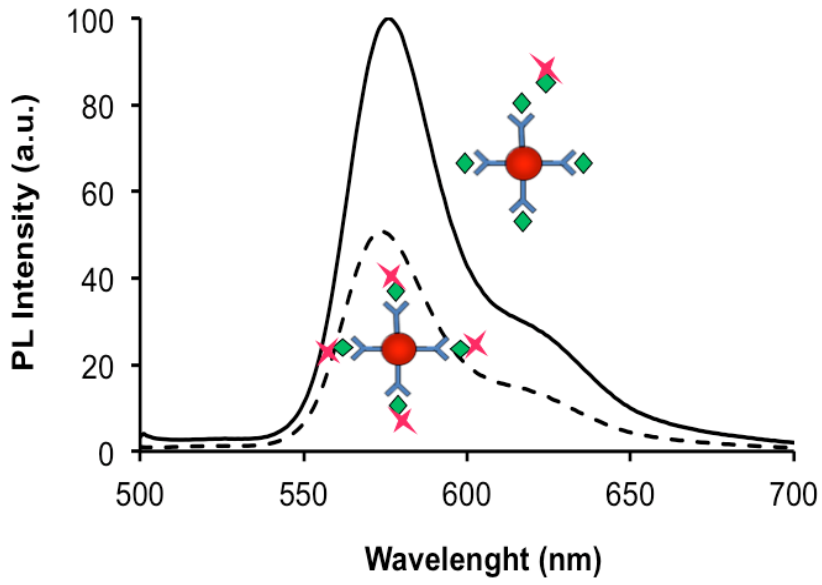


**Figure 2** - The photoluminescence intensity of Cy3B in the presence of AuNP-antibody conjugates is low due to quenching by the AuNPs (dashed trace). When the *Pf*Hsp70 antigen (analyte) binds to the AuNP-antibody conjugates, an increased amount of Cy3B-labeled *Pf*Hsp70 is free in solution causing an increase in the photoluminescence intensity (solid trace). Red circles are AuNPs, blue "Y" are antibodies, green diamonds represent the antigen, and pink stars represent the Cy3B label.

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