academicJournals

Vol. 13(8), pp. 978-982, 19 February, 2014 DOI: 10.5897/AJB10.723 ISSN 1684-5315 ©2014 Academic Journals http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Light microscopic detection of *Plasmodium falciparum* in vitro through *Pf* histidine rich protein 2 (HRP 2) gold conjugate labeling: Rapid diagnosis of cerebral malaria in humans

Ogundele Olalekan Michael^{*1}, Saheed Sabiu², Adekeye Adeshina Oloruntoba¹, Adeniyi Philip Adeyemi¹, Ogedengbe Oluwatosin Olalekan¹, Enye Linus Anderson¹ and Anosike Victor Ikenna³

Accepted 15 June, 2012

Plasmodium falciparum (Pf) has been found to be the deadliest of all the known species of the parasite capable of infecting humans; this is because it is capable of causing severe cerebral tissue damage. This study was carried out to demonstrate the parasite in the host blood *in vitro* through immunogold labeling using antibodies against *Plasmodium falciparum* histidine rich protein 2 (HRP 2); a major metabolite released during the cause of the parasite infection and feeding in the erythrocyte. 12 known *Pf* positive samples were obtained from across the six geopolitical zones of Nigeria and were further characterized by Geimsa thick and thin film for parasite identification parasite count expressed as parasites/μl of blood. An average of 400 parasites/μl of blood was obtained in each of the samples used for this study. *Pf*-HRP 2 antibody was conjugated to freshly prepared colloidal gold of particle size 40 nm. The conjugation process was blocked with bovine serum albumin (BSA) and the conjugate itself preserved by 1% glycerol and 0.01% sodium azide. The parasite count was titrated against the *Pf*-HRP 2 gold conjugate and was analyzed under the light microscope with a fluorescent filter. Reactivity and specificity of *Pf*-HRP 2 gold conjugate was found to be highly specific and gave direct identification of the erythrocytes infected with the parasite. A good contrast was also obtained between uninfected erythrocytes, parasite and the infected erythrocytes.

Key words: Plasmodium falciparum, malaria, fluorescent microscope, HRP 2, antibody, gold conjugates.

INTRODUCTION

Malaria is a dominant infection in the tropics, especially in Africa, South East Asia and the Amazon of South

America, this so because these regions provide suitable climate conditions for the vector to survive and reproduce

*Corresponding author. E-mail: ola.ogundele@abuad.edu.ng. Tel: +2347031022702.

Abbreviation: Pf -HRP 2, Plasmodium falciparum histidine rich protein 2.

¹Department of Anatomy, College of Medicine and Health Sciences, Afe Babalola University, Ekiti State Ado-Ekiti, Nigeria.

²Department of Biotechnology and Molecular Biology, Faculty of Pure and Applied Sciences, Kwara State University, Ilorin, Nigeria.

³Department of Anatomy, College of Medicine, Bingham University, Karu, Nassarawa State, Nigeria.

(the vector being female anopheles mosquito) (Fukuda et al., 2009). The parasite in the host system binds to the endothelium of cerebral vessels in the brain; the binding process is called cyto-adherence and it involves knob formation (Mayer et al., 2012). Accumulation of infected erythrocyte in these blood vessles thus prevents exchange of nutrients in the capillary bed, leading to a state of ischemia or anoxia (Hora et al., 2009; Nacer et al., 2011). The tissues of the brain are supplied in segmented tissue blocks of about 1 mm³, such that a tissue block will correspond to the region solely supplied by a terminal artery. Terminal arteries are end arteries that do not branch or anastomose; therefore shortage of blood supply cannot be compensated for eventually causing avascular necrosis primary to cerebral symptoms and hemorrhage (Willie et al., 2012).

In view of this, rapid diagnosis of falciparum infection is important. To detect plasmodium falciparum and other species of the parasite, several proteins have been identified as suitable indictors of the presence of such parasite in the blood stream (Hansen et al., 2012). Examples of such proteins include plasmodium aldolase. plasmodium lactate dehydrogenase and plasmodium histidine rich protein 2 (HRP 2) (Waisberg et al., 2012). Histidine rich protein 2 has been described as being the most effective in detecting falciparum infection (Grobusch et al., 2003). A major challenge of rapid diagnostics using in vitro kits is that the HRP 2 remains in the blood stream after the parasite has cleared post treatment with an antimalaria molecule, thus giving false results (Grobush et al., 2003). The World health organization (WHO) recommends microscopy as the standard for diagnosis of malaria and identification of parasite because of the various limitations of the rapid diagnostic tests (RDTs) (prozoning effects, false positive and cross reactions) (Cojoc et al., 2012). In this technique we have brought together in the same context the principle of microscopy and the biological techniques of in vitro test kits as a fast method in which Pf infected parasites can be guickly identified. This method utilizes the microscopic properties of nanoparticles (colloidal gold) and its ability to serve as a fluorescent agent and a tool for microscopy.

MATERIALS AND METHODS

Preparation of colloidal gold and gold conjugate

1% sodium citrate solution was made by dissolving 1 g of sodium citrate (Sigma) in 100 ml of deionised water (Millipore, France) the water was tested for salts and have a pH of 6.0. 2000 ml of the water was placed in a conical flask and 50 ml of 1% gold (99% pure from Sigma) chloride was added. A stirrer magnet covered with a tephlon was inserted into the solution, the hot plate was switched on such that the temperature was set at 70°C, a conical flask, 250 ml capacity was inverted to cover the opening of the larger conical flask in order to prevent loss of water by vaporization. The solution was allowed to boil and the stop watch was started, until the colour

changes from red to purple (approximately 1 min). 9.6 ml of 1% gold chloride was added and the colour again changed from red to purple, 2 ml 0.7% gold chloride was then added as the colour was observed to have changed from purple to pink. The solution was allowed to cool by constantly running water on the cone region of the conical flask. The colloidal gold was analyzed using the spectrophotometer in the spectrum region, and the peak absorbance recorded was 528 nm. The particle size as analyzed under the fluorescent microscope was approximately 40 ± 1 nm, modifying the methods of Han et al. (2012).

Conjugation of gold with Pf-HRP 2 antibody (monoclonal)

The Tris HCl was poured into a beaker and the antibody was added at a concentration of 5 mg/L, the colloidal gold, twice the volume of the buffer, was the added and the solution was blocked with 1.5% bovine serum albumin (BSA, Sigma), added as 10% solution, 0.05% sodium azide (Sigma) was added to prevent microbial infection and 1% glycerol was then added to preserve the conjugate. The solution was centrifuged at 10000 rpm and 4°C, the supernatant was collected and discarded while the final conjugate particle size and concentration was determined, using the spectrophotometer. The colloidal gold and gold conjugate samples were profiled in a hollow slide under the fluorescent microscope (Han et al., 2012; Tsai et al., 2012).

Incubation of gold conjugates and blood samples

100 μ l of the gold conjugate was obtained in an eppendorf and mixed with 10 μ l of the sample. The samples were mixed by gently shaking the eppendorf tube, and was incubated at 37°C temperature for 15 min, 0.05% of phytoheamoagglutinin (PHA) was added to partially lyse the red blood cell (RBC). The mixture was then smeared on a hollow slide and viewed under the fluorescent microscope and compared with a normal smear of Pf-HRP 2 conjugate. The gold conjugate was also used in soaking a conjugate pad and assembled into a rapid diagnostic kit on a nitrocellulose membrane coated with anti HRP 2 antibody to allow for a double sandwich immunoassay.

RESULTS AND DISCUSSION

The colloidal gold particles can be seen as dots (otherwise quantum dots) in the light microscope while the curves of the spheres can give fluorescence with a filter in place (green fluorescence filters were used in Zeiss Primo Star light microscope, magnification ×1000) (Figures 1A and B). Arrow heads in Figure 2A shows the symmetrical attachment of the tail of the Y shaped antibody leaving the epitope regions (the free ends of the Y) directed at right angles from the surface of the spheres. In this study, approximately four antibody proiections were observed per colloidal gold in focus. Arrow heads in B shows the Y shaped slender free ends of the antibodies (magnification ×1000). In Figure 3, colloidal gold (c), parasite (p) and labeled parasite (f) were embedded in the erythrocyte. F1 represents an advanced stage of parasite infection while F2 represents the late

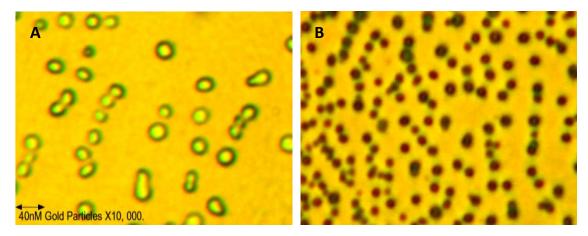


Figure 1. 40 nm colloidal gold under light microscope with fluorescence filters, this is to demonstrate in the first instance that colloidal gold can be viewed without enhancement under the light microscope (B), and with fluorescence filters in the same microscope in (A) (magnification ×1000).

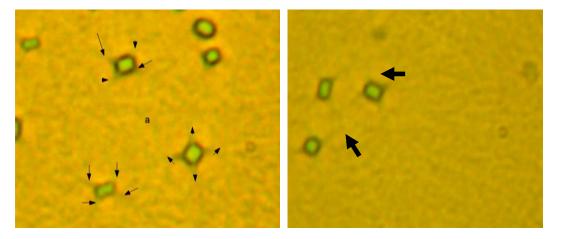


Figure 2. Demonstration of colloidal gold conjugated with Pf-HRP 2 using the fluorescence filters (magnification $\times 1000$).

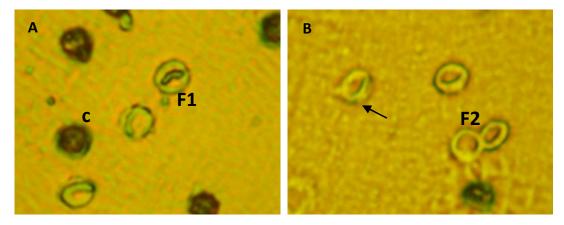


Figure 3. *Pf* infected erythrocytes shown under fluorescent filter in light microscopy (magnification ×1000). C, Colloidal gold; F, labeled parasite.

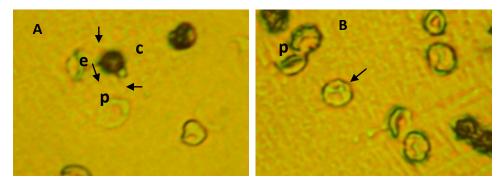


Figure 4. Demonstration of the specificity of *Pf*-HRP 2 to the antibody and the parasite in the infected erythrocyte (magnification ×1000). c, Colloidal gold; p, parasite; e, erythrocyte.

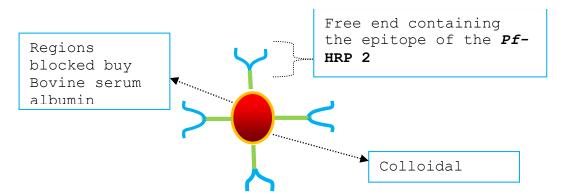


Figure 5. Schematic illustration of the multiplanar appearance of *Pf*-HRP 2 gold conjugate (Courtesy: Trinitron Biotech Limited, Nigeria).

and multiplication stage of the infection (magnification ×1000). Arrow heads in Figure 4A shows the projecting free limbs of the antibodies, colloidal gold (c), parasite (p) and erythrocyte (e). Figure 4B shows parasite localized in infected erythrocytes at different stages of the infection (magnification ×1000).

In living tissues, colloidal gold can be used to study molecular traffic and transport system. When labeled with appropriate antibodies, they localize in specific regions of the cell (Nolan et al., 2012). Thus, this technique was designed to localize the colloidal gold on the surface of erythrocytes and in the cytoplasm of cells housing the parasite in the blood and also free parasites in the blood. Considering the electrical properties of the surface of colloidal gold as being positively charged, and the localization of chains of negatively charged amino acids in the tail of the Y shaped antibodies (also *Pf*-HRP 2), an electrostatic force of attraction can occur between colloid-dal gold particles and the antibodies (Gao et al., 2012).

A major advantage of this technique is that it leaves the reactive part of the antibody unbounded such that they remain as free edges of the multiplanar gold conjugate with two epitope per one Y shaped antibody and possessing four of such planar structures.

The planar structure as described in Figure 5 gives the colloidal gold conjugate stability and shows that aggregation has not occurred during the preparation of the gold conjugate. This technique has eliminated the false positive observed post treatment; when the specimen is incubated with the gold conjugate, it will show that the protein is present but the parasite is absent, thus breaking the tie for a false positive malaria test post treatment. It will also aid quick counting of parasite in the blood, as it tags both parasite and parasite infected cells, helping in diagnosis and treatment.

This feature is also of advantage over the RDTs, as the colour formation (chromoagregation of colloidal gold conjugates) will most likely give a uniform coloration, from 750 parasites/ μ l till 3000 parasites/ μ l of blood. In this study, it is observed that the parasites are specifically labeled and can be counted on the slide to give a definitive count, by locating bounded conjugates. Since this technique utilizes antibody, it will be of advantage in identifying parasites microscopically; antibody-antigen

reactions are specific thus giving the advantage of specifically identifying plasmodium falciparum even at lower magnifications. This technique can be employed for a wide range of blood infections including determination of the extent of spread of cancer cells. It can be used to target infection sites for live studies in tissues and to treat cancers, as the conjugated colloidal gold can be irradiated to explode tropically within tumor cells thus destroying the cancers.

REFERENCES

- Cojoc D, Finaurini S, Livshits P, Gur E, Shapira A, Mico V, Zalevsky Z (2012). Toward fast malaria detection by secondary speckle sensing microscopy. Biomed. Opt. Express. 3(5):991-1005.
- Fukuda MM, Klein TA, Kochel T, Quandelacy TM, Smith BL, Villinski J, Bethell D, Tyner S, Se Y, Lon C, Saunders D, Johnson J, Wagar E, Walsh D, Kasper M, Sanchez JL, Witt CJ, Cheng Q, Waters N, Shrestha SK, Pavlin JA, Lescano AG, Graf PC, Richardson JH, Durand S, Rogers WO, Blazes DL, Russell KL; AFHSC-GEIS Malaria and Vector Borne Infections Writing Group, Akala H, Gaydos JC, DeFraites RF, Gosi P, Timmermans A, Yasuda C, Brice G, Eyase F, Kronmann K, Sebeny P, Gibbons R, Jarman R, Waitumbi J, Schnabel D, Richards A, Shanks D (2011). Malaria and other vector-borne infection surveillance in the U.S. Department of Defense Armed Forces Health Surveillance Center-Global Emerging Infections Surveillance program: Review of 2009 accomplishments. BMC Public Health. 2011 Mar 4; 11 Suppl 2:S9.
- Gao J, Huang X, Liu H, Zan F, Ren J (2012)Colloidal stability of gold nanoparticles modified with thiol compounds: bioconjugation and application in cancer cell imaging. *Langmuir*. 28(9):4464-4471.
- Grobusch MP, Hänscheid T, Göbels K, Slevogt H, Zoller T, Rögler G, Teichmann D (2003). Comparison of three antigen detection tests for diagnosis and follow-up of falciparum malaria in travellers returning to Berlin, Germany. Parasitol. Res. 89(5):354-357.
- Han KC, Yang EG, Ahn DR (2012). A highly sensitive, multiplex immunoassay using gold nanoparticle-enhanced signal amplification. Chem. Commun. (Camb). 48(47):5895-5897.
- Hansen KS, Pedrazzoli D, Mbonye A, Clarke S, Cundill B, Magnussen P, Yeung S (2012). Willingness-to-pay for a rapid malaria diagnostic test and artemisinin-based combination therapy from private drug shops in Mukono district, Uganda. Health Policy Plan. 2012 May 15.

- Hora R, Bridges DJ, Craig A, Sharma A (2009). Erythrocytic casein kinase II regulates cytoadherence of Plasmodium falciparum-infected red blood cells. J. Biol. Chem. 284(10):6260-6269.
- Mayer C, Slater L, Erat MC, Konrat R, Vakonakis (2012) Structural analysis of the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) intracellular domain reveals a conserved interaction epitope. J. Biol. Chem. 287(10):7182-7189.
- Nacer A, Roux E, Pomel S, Scheidig-Benatar C, Sakamoto H, Lafont F, Scherf A, Mattei D. (2011). Clag9 is not essential for PfEMP1 surface expression in non-cytoadherent Plasmodium falciparum parasites with a chromosome 9 deletion. PLoS One2011, 6(12):e29039.
- Nolan JP, Duggan E, Liu E, Condello D, Dave I, Stoner SA (2012). Single cell analysis using surface enhanced Raman scattering (SERS) tags. Methods. 57(3):272-279.
- Tsai DH, Elzey S, Delrio FW, Keene AM, Tyner KM, Clogston JD, Maccuspie RI, Guha S, Zachariah MR, Hackley VA (2012) Tumor necrosis factor interaction with gold nanoparticles. Nanoscale. 4(10):3208-3217.
- Waisberg M, Cerqueira GC, Yager SB, Francischetti IM, Lu J, Gera N, Srinivasan P, Miura K, Rada B, Lukszo J, Barbian KD, Leto TL, Porcella SF, Narum DL, El-Sayed N, Miller LH, Pierce SK (2012) A highly sensitive aptasensor towards Plasmodium lactate dehydrogenase for the diagnosis of Malaria. Proc. Natl. Acad. Sci. U.S.A. 109(14):5429-5434.
- Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA, Ainslie PN (2012). Regional Brain Blood Flow in Man during Acute Changes in Arterial Blood Gases. J. Physiol. 590(Pt 14):3261-3275.