Development of a novel Malaria Antibody assay utilizing antigens from all 5 human pathogenic Plasmodium species

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Introduction

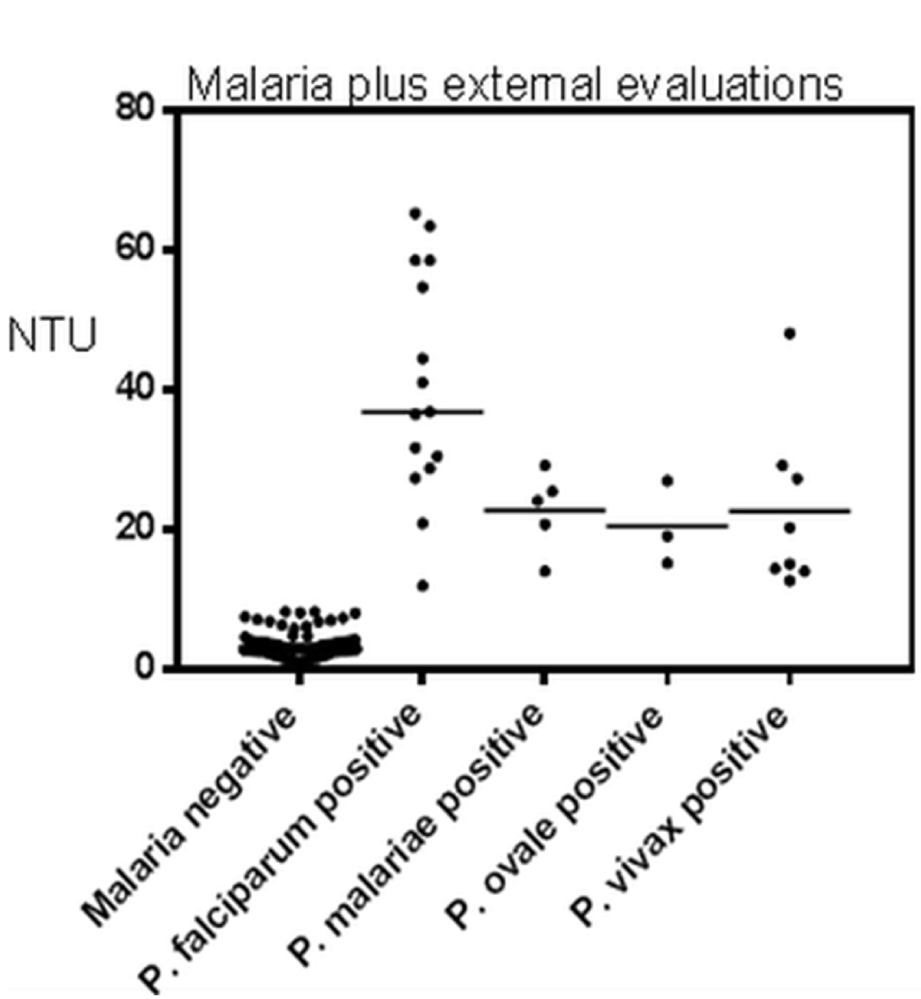
The proper diagnosis of Malaria disease is essential to provide early treatment and improve the prognosis of patients. Serological methods often fail in diagnosing new borns due to their altered immune system, resulting in a need for new diagnostic methods reliably working with sample from patients ranging from 0-12 month of age. Transfusion-transmitted Malaria is rare, but it may produce severe problems in the safety of blood transfusion and blood related products due to the lack of reliable procedure to evaluate donors potentially exposed to malaria. Microscopy, still considered the gold standard for diagnosing malaria. It is time consuming and requires trained expertise. Microscopy has a limited use in blood banking and screening of populations.

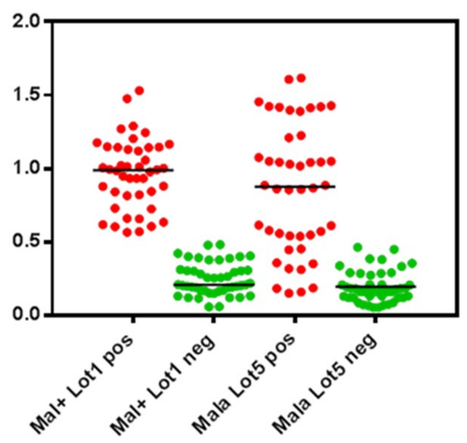
ELISAs are known to be ideal for high throughput screening with high sensitivity and specificity, but it also requires trained personal and an equipped laboratory. Lineblots are often used as confirmatory tests since they provide high sensitivity and specificity. There is nearly no lab equipment needed to perform this kind of assay. In addition, blots can also be used in automated processes for high throughput screening.

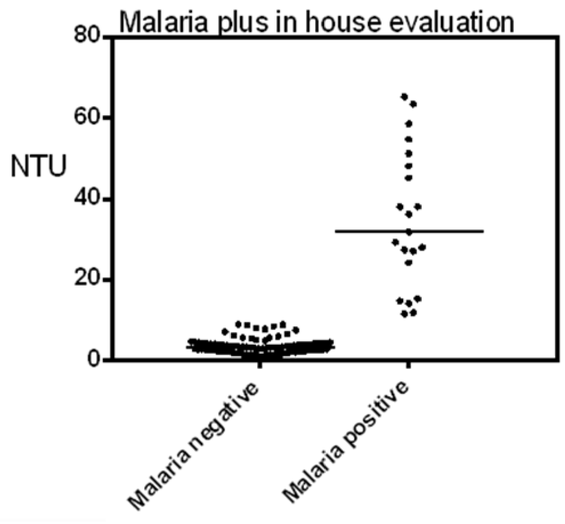
Here we show an improved diagnostic performance of the new antibody detection Systems (ELISA and Lineblot) utilizing early and late antigens of all 5 human pathogenic Plasmodium species (*P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*) compared to test systems only relying on antigens derived from one or two Plasmodium species.

The novel Lineblot is able to discriminate between all 5 parasite species.

Assays with a limited number of antigens often fail to detect antibodies from certain regions of the world. For evaluation purpose, we collected samples from all over the world, including samples from newborns. We evaluated the performance of ELISA and Lineblot directly in endemic countries with samples of patients who presented symptoms akin to malaria infection in local hospitals.







Diagnostic Sensitivity: 97,00 % (130/134)
Diagnostic Specificity: 96,50 % (301/312)
Agreement: 96,60 % (431/446)

Results

The first Malaria Antibody ELISA developed by NovaTec Immundiagnostica GmbH was based on antigens derived from *P. falciparum* and *P. vivax* only. While this assay showed an excellent diagnostic performance with samples obtained from most regions of the world (e.g. Africa, South America, Asia) it showed some drawbacks with certain samples originated from certain regions of south-east Asia (e.g. Korea), especially with samples not positive for *P. falciparum* or *P. vivax*. For this reason we went back to the antigen design and saw great differences within the amino acid sequence of MSP1 and CSP between all human pathogenic Plasmodium species (see Fig.1-3). Muerhoff et al., described already in 2010 that commercial kits fail to detect Malaria caused by certain Plasmodium species in case that a species specific antigen was missing within the assay. For this reason we developed, produced and purified MSP1 and CSP antigens of all 5 human pathogenic Plasmodium species. After DNA synthesis and expression of the novel antigens we compared the performance of diagnostic assays with P. falciparum and P. vivax antigens alone against assays utilizing antigens derived from all 5 human pathogenic Plasmodium species. Evaluation with randomly selected samples showed a better discrimination of positive and negative samples of the improved version of the kit compared to the old version (see Fig 4). For a more “in depth” evaluation of the new assay we have had a look on European blood donors as negative matrix, samples from patients positive for Chagas, Leishmania and Toxoplasma as potential cross-reactive matrix and samples from Malaria positive patients defined to species level. Specificity according to blood bank samples was >95%. No cross-reaction with samples of Chagas, Leishmania and Toxoplasma could be observed. In addition the sensitivity for all Plasmodium species was >95% (see Fig. 5). The old Malaria assay was tested for its ability to detect congenital transfer of Malaria from the mother to the baby. Residual samples of this investigation were used to evaluate the new 5 antigen Malaria assay for the ability to detect antibodies in new borns. Like the old assay also the new assay was able to detect anti Plasmodium antibodies in Malaria positive babies.

Diskussion

The new Malaria Antibody ELISA utilizing antigens from all 5 human pathogenic Plasmodium species shows great advantages compared to the old Malaria Antibody ELISA just using antigens from Pl. Falciparum and P. vivax. Sensitivity increased with rare Plasmodium species compared to assays just using 1-2 antigens. Furthermore a better differentiation between positive and negative results was observed when looking on regular samples. Specificity remained the same. Also no cross-reactions could be observed with the new Malaria assay. Next steps are the development of a multiplex Lineblot to differentiate between different species as well as a lateral-flow rapid test for in-field usage.

Submitted: “Sensitivity and Specificity of a new ELISA kit which uses recombinant antigens to Plasmodium falciparum and vivaxfor the detection of malaria antibodies in Cameroon”; Longdoh A Njunda, Andreas Latz, Bita D Tayong and Tebit E Kwenti

In preparation: “Evaluation of ELISA-based NovaLisa test kit for malaria diagnosis in an endemic area of Thailand”; Wanna Chaijaroenkul and Kesara Na-Bangchang