

**The Afro-Immuno Assay Multi-Centre
Network Project and Capacity Building for
Malaria Vaccine Development In Africa**

AN AMANET SPONSORED NETWORK

Background

- **Antigen-specific antibody-mediated immune responses play a role in natural protection against clinical malaria**
- **Conflicting estimates of this association have emerged from studies in different geographical settings**
- **May be due to the use of different methodologies.**

REASONS

- 1. Reagents**
- 2. Study design**
- 3. Immunological assay**
- 4. Statistical methodology**
- 5. Differences in transmission**

To render such studies more comparable, the Afro-Immuno Assay (AIA) network project was initiated

**The overall aim of the multi-centre network project is:
To develop and introduce standardized immunological assays that could:**

- 1. form part of a set of criteria for the validation of promising malaria vaccine candidate antigens in pre-clinical immuno-epidemiological studies**
- 2. will be used for immunogenicity assessment in clinical trials and provide essential baseline data that allows for comparisons between trials**
- 3. will enhance quality assured laboratory capacity and capability**

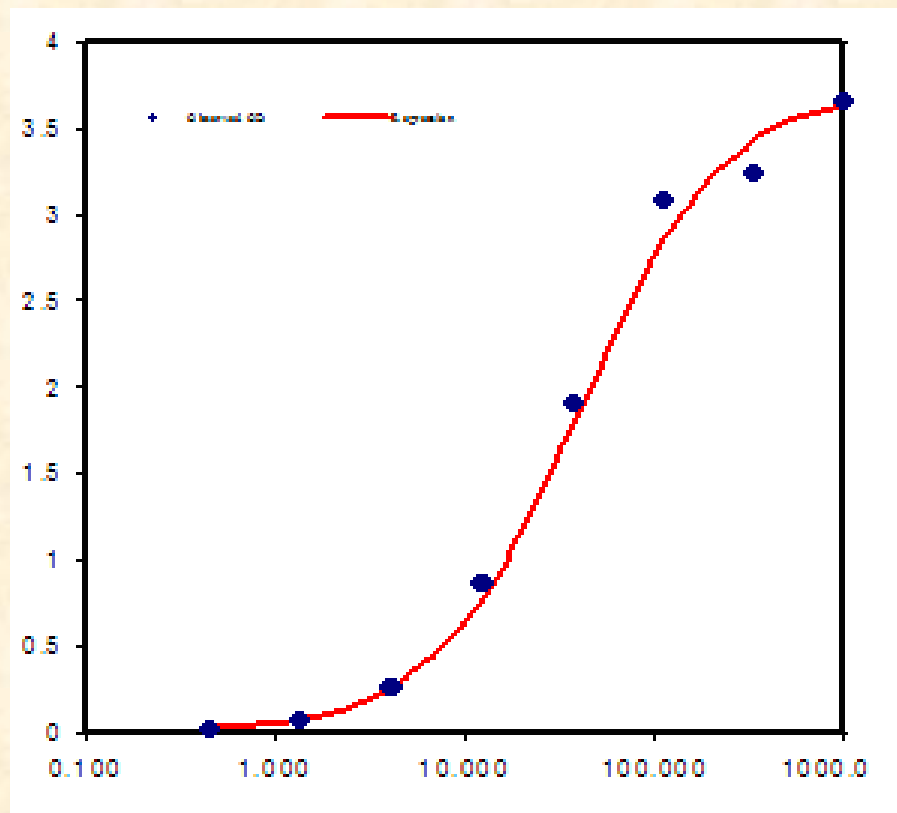
- **AIA phase 1 included, 6 African and 3 European Institutions**
- **AIA developed and validated standardized ELISA**
- **The AIA ELISA was used to assess the relationship between antibody responses against 4 potential malaria vaccine candidate antigens (AMA1, MSP1-19, MSP3, GLURP) and protection from clinical malaria.**

OPTIMIZATION/VALIDATION OF AIA ELISA

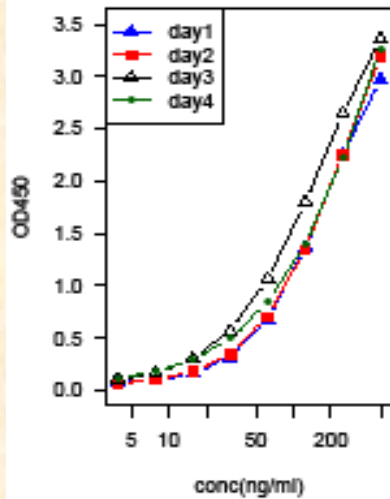
- 1. Antigen coating: GLURP, MSP3, MSP1, AMA-1**
- 2. Dilution of subclass-specific antibodies**
- 3. Cross-reactivity of subclass-specific antibodies**
- 4. Stability of antigen coated plates**
- 5. Stability of serum dilutions**
- 6. Reproducibility of the assays**

Standard curves for conversion ELISA OD to antibody units (AU)

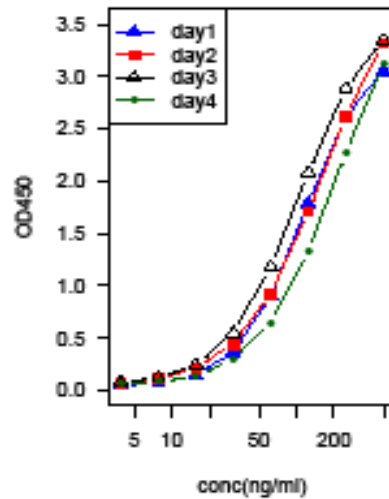
Standard Curve			
Ref: ng/ml	OD - Blk	OD Pred	Include
1000.0	3.654	3.648	<input checked="" type="checkbox"/>
333.3	3.240	3.432	<input checked="" type="checkbox"/>
111.1	3.079	2.847	<input checked="" type="checkbox"/>
37.0	1.898	1.784	<input checked="" type="checkbox"/>
12.3	0.867	0.775	<input checked="" type="checkbox"/>
4.1	0.258	0.260	<input checked="" type="checkbox"/>
1.4	0.073	0.079	<input checked="" type="checkbox"/>
0.5	0.021	0.023	<input checked="" type="checkbox"/>



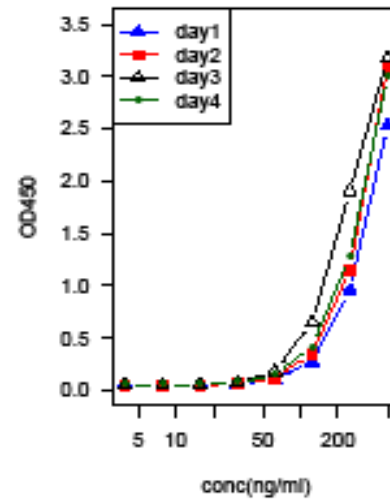
Gh GLURP-IgG



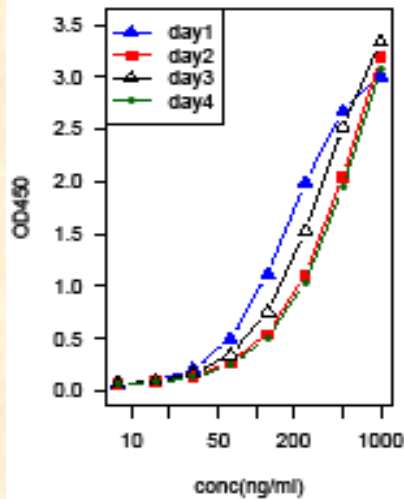
Gh GLURP-IgG1



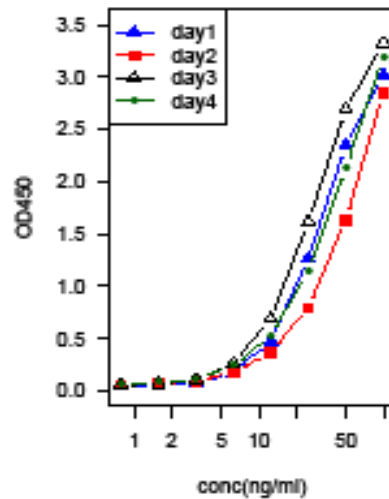
Gh GLURP-IgG2



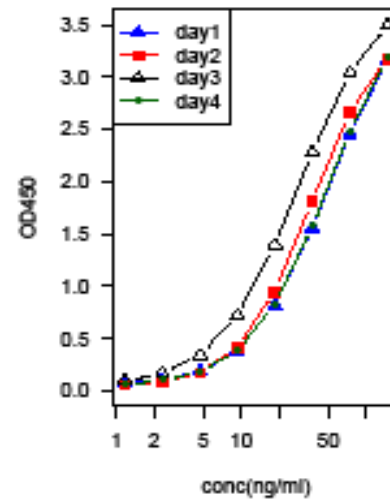
Gh GLURP-IgG3



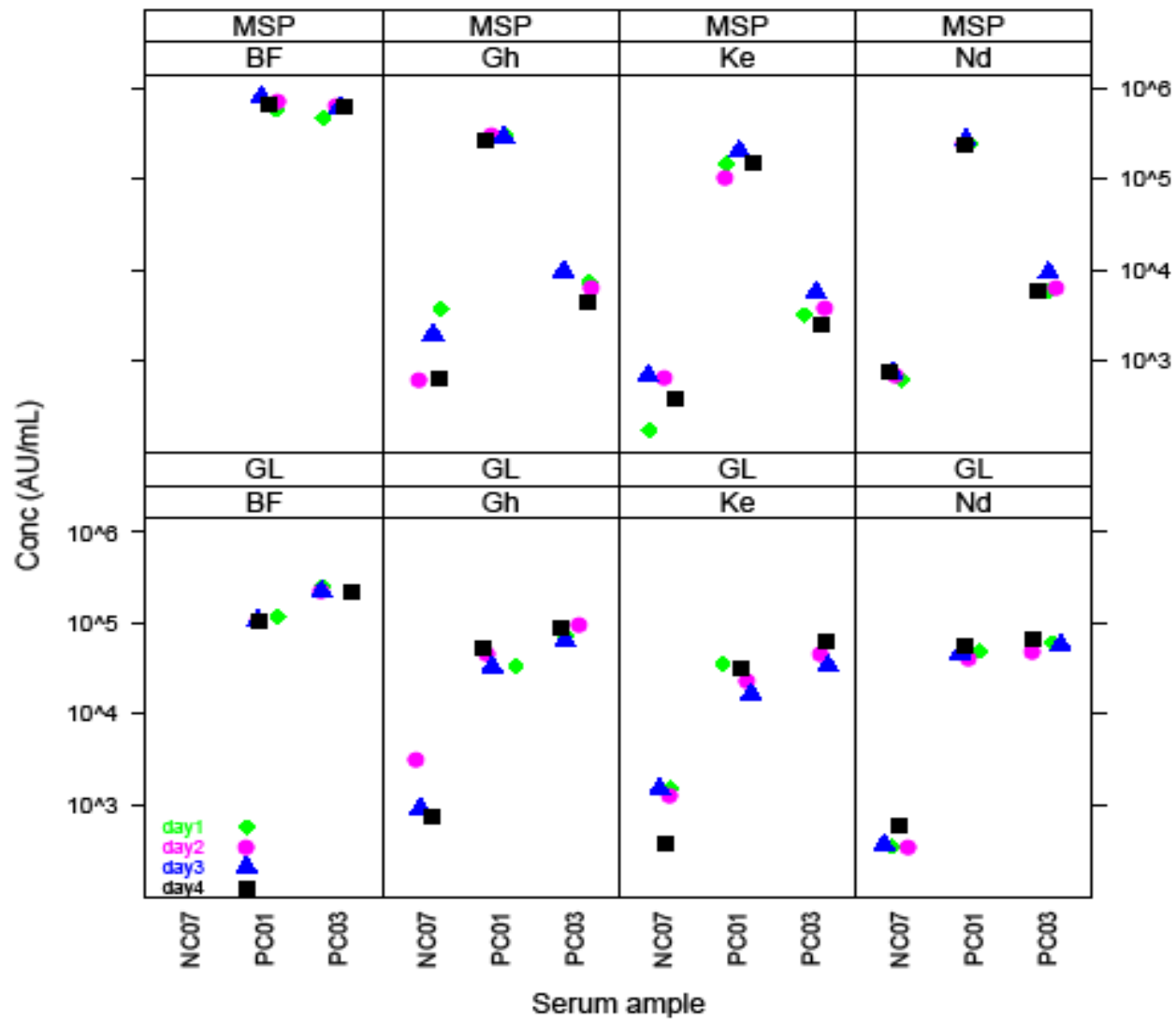
Gh GLURP-IgG4



Gh GLURP-IgM



Total IgG



RESULTS

Data from two of the study sites;

Ghana showed levels MSP1-19

IgG1 [(0.80 (0.67–0.97), $p = 0.018$)]

IgM [(0.48 (0.32–0.72), $p < 0.001$)]

correlated with protection from malaria in Ghanaian children

while for Burkina Faso:

IgG1 to AMA1 [0.87 (0.78–0.97), $p = 0.013$]

and IgG3 to GLURP [0.82 (0.72–0.94), $p = 0.004$] were associated with reduced risk to malaria in Burkinabe children.

CONCLUSIONS

- **Antibodies to MSP1-19, GLURP, AMA1 shown to independently correlate with reducing the risk of clinical malaria in Ghanaian and Burkinabe children**
- **Substantiating their potential as malaria vaccine candidates.**
- **The differing conclusions in the two studies may be due to the differences in malaria transmission**
- **Functional assays are required to confirm if these associations reflect functional roles of antibodies that correlated with protection from clinical malaria.**

The AIA ELISA has been used:

1) in pre-clinical malaria immuno-epidemiological studies

a) Cohort study of the association of antibody levels to AMA1, MSP1-19, MSP3 and GLURP with protection from clinical malaria in Ghanaian children. *Dodoo et al, Malar J. 2008 Jul 29;7:142.*

b) Humoral responses to Plasmodium falciparum blood-stage antigens and association with incidence of clinical malaria in children living in an area of seasonal malaria transmission in Burkina Faso, West Africa.

Nebie et al, Infect Immun. 2008 Feb;76(2):759-66.

c) Cytophilic antibodies to *Plasmodium falciparum* Glutamate Rich Protein are associated with malaria protection in an area of holoendemic transmission. *Lusingu et al, Malar J. 2005 Sep 29;4:48.*

2) As well as in malaria vaccine trials:

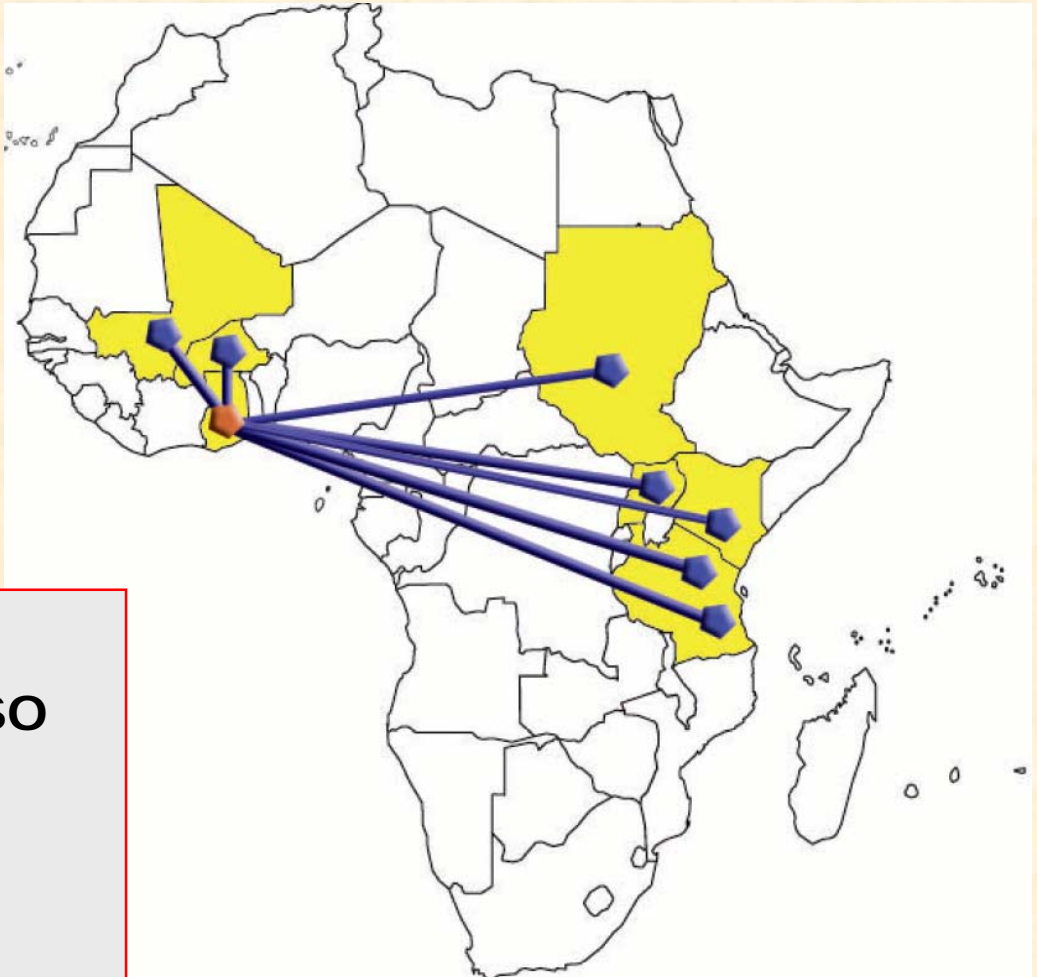
a) Humoral and cell-mediated immunity to MSP3 peptides in adults immunized with MSP3 in malaria endemic area, Burkina Faso
Nebie et al, Parasite Immunol. 2009 Aug;31(8):474-80.

b) Satisfactory safety and immunogenicity of MSP3 malaria vaccine candidate in Tanzanian children aged 12–24 months
Malaria Journal 2009, 8:163

**AIA ELISA methodologies are available at
AMANET website: <http://www.amanet-trust.org/>**

AIA Phase 2

- 8 African+2 European Institutions/sites
- New Antigens
- Data management
- Statistical support
- 1 Masters student per site
- 1 PhD student in Statistical methodology



1. NMIMR - GHANA
2. CNRFP – BURKINA FASO
3. MRTC- MALI
4. KEMRI - KENYA
5. IEND- SUDAN
6. MAKERERE- UGANDA
7. KCMC - TANZANIA
8. NIMR-Tanga - TANZANIA
9. SSI - DENMARK
10. BPRC- NETHERLANDS

Better designed Cohort Studies with:

- 1) adequate consideration of potential confounders**
- 2) impact of the short lived malaria induced antibodies**
- 3) differentiate the exposure specific antibodies from those related to protection**
- 4) 4 sites undertake T=Cell assay in relation to immunity to malaria**
- 5) 4 sites undertake functional assays to assess the quality of immune responses**

**AMANET, DGIS, EMVDA, EDCTP
are duly acknowledged for
financial support that has made
the South-South and North-
South Afro-Immuno Assay
Multicentre Network Project
successful**

THANKS