Measuring Antibody Avidity To Plasmodium Falciparum Merozoite Antigens Using A Multiplex Immunoassay Approach

CURRENT STATUS: UNDER REVISION

Malaria Journal BMC

Diane Wallace Taylor
University of Hawai'i at Manoa John A Burns School of Medicine

✉ dwtaylor@hawaii.edu *Corresponding Author*
ORCID: <https://orcid.org/0000-0002-0988-4290>

Naveen Bobbili
University of Hawaii

Alexander K Kayatani
University of Hawaii

Samuel Tassi-Yunga
University of Hawaii

Rose FG Leke
Universite de Yaounde I

DOI:

10.21203/rs.2.23647/v1

SUBJECT AREAS

Infectious Diseases

KEYWORDS

Malaria, Plasmodium falciparum, antibodies, antibody avidity, multiplex immunoassay, methods study

Abstract

Background: Antibodies (Ab) play a significant role in immunity to *Plasmodium falciparum* malaria.

Usually, following repeated exposure to pathogens, affinity maturation and clonal selection take place, resulting in increased antibody avidity. However, some studies suggest affinity maturation may not take place to malaria antigens in endemic areas. Information on development of antibody avidity is confusing and conflicting, in part, because different techniques have been used to measure avidity. Today, bead-based multiplex immunoassays (MIA) are routinely used to simultaneously quantitate antibody levels to multiple antigens. This study evaluated the feasibility of developing an avidity MIA with 5 merozoite antigens (AMA1, EBA-175, MSP1-42, MSP2, MSP3) that used a single chaotropic concentration. Methods: The most common ELISA protocols that used the chaotropic reagents guanidine HCl (GdHCl), urea, and ammonium thiocyanate (NH₄SCN) were adapted to a multiplex MIA format. Then, different concentrations of chaotropes and incubation times were compared and results were expressed as an Avidity Index (AI), i.e., percentage of antibody remaining bound in the presence of chaotrope. Experiments were conducted to i) identify the assay with the widest range of AI (discriminatory power), ii) determine the amount of chaotrope needed to release 50% of bound Ab using plasma from adults and infants, and iii) evaluate assay repeatability. Results: Overall, 4M GdHCl and 8M urea were weaker chaotropes than 3M NH₄SCN. For example, they failed to release significant amounts of Ab bound to MSP1-42 in adult plasma samples; whereas, a range of AI values was obtained with NH₄SCN. Titration of NH₄SCN revealed that 2M urea gave the widest range of AI for the 5 antigens. Binding studies using plasma from 40 adults and 57 one-year old infants in Cameroon showed that $2.1\text{M} \pm 0.32$ (mean $\pm$ SD) NH₄SCN (adults) and $1.8\text{M} \pm 0.23\text{M}$ released 50% of bound Ab from the merozoite antigens. The final avidity multiplex assay was highly repeatable. Conclusions. An avidity MIA is feasible for the 5 merozoite antigens that uses a single concentration (2M) of NH₄SCN. The assay provides a simple method to quickly obtain information about Ab quantity and quality in the acquisition of immunity to malaria in endemic populations.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

Figures

Figure 1

Figure 1

Comparison of Avidity Indexes for 5 Merozoite Antigens using 3 different Chaotropic Agents. Antigen-coupled beads were incubated with a 1:600 dilution of the positive control (PC) and plasma from 5 individual adults (1# - #5) living in a high transmission area in Cameroon. After incubation, Ab-bound beads were incubated with 4M GdHCl for 10 minutes, 8 M urea for 10 minutes, or 3M NH₄SCN for 30 minutes. Results show mean AI values (i.e., the percentage of Ab remaining bound after treatment with salt) $\pm$ SD for 3 replicate samples. Antigens included: AMA1 3D7, EBA-175, MSP1 (3D7), MSP2 (FC27) and MSP3. Baseline (no salt) MFI values [mean $\pm$ SD] for the 6 plasma samples (PC + 5 adults) were as follows: A) AMA1= 23,677 $\pm$ 1079 MFI; B) EBA-175=22,002 $\pm$ 1573 MFI; C) MSP1= 11,196 $\pm$ 1405 MFI; D) MSP2= 5,702 $\pm$ 2282; and E) MSP3= 4,128 $\pm$ 1232 MFI.

Figure 2:

Figure 2

Avidity Indexes after Treatment with 8M urea and 1M, 2M and 3M NH₄SCN. The same PC and samples from 5 individual adults used in Figure 1 were re-tested in this experiment.

Following incubation of plasma with the antigen-coupled beads, the complexes were incubated with 8M urea or 1M, 2M and 3M or NH₄SCN for 30 minutes and amount of Ab remaining bound (AI) were calculated. Error bars represent the mean $\pm$ one SD for 3 replicates. Baseline MFI values [mean $\pm$ SD based on 15 replicates in the absence of salt] were as follows: A) AMA-1 23,958 $\pm$ 98 MFI; B) EBA-175: 22,413 $\pm$ 166; C) MSP1 3D7: 10,169 $\pm$ 208 MFI; D) MSP3: 2,122 $\pm$ 82MFI.

Figure 3:

Figure 3

Comparison of the Length of Incubation with the Chaotrope. Antigen-coupled beads were incubated with the pooled Positive Control (PC) for 60 minutes. Beads were then washed,

incubated with 4M GdHCl, 8M urea, or 1M, 2M or 3M NH₄SCN for either 15 or 30 minutes at RT on a rotary shaker. Following washing and incubation with R-phycoerythrin-labeled, Goat anti-human IgG (Fc fragment) for 60 minutes, MFI were recorded and AI calculated. Error bars represent mean + SD based on 3 replicates. Baseline MFI values [mean $\pm$ SD MFI for 30 replicates in the absence of salt] were as follows: AMA-1 23,955 $\pm$ 133 MFI; EBA-175 22,473 $\pm$ 587; MSP1 (3D7) 10,042 $\pm$ 333 MFI; and MSP3: 1,990 $\pm$ 155 MFI.

Figure 4.

Figure 4

Variation among Replicates Assays. The Positive-Control (VPC) and plasma from two adults (P2/064, P2/87) were used as positive controls on 13 plates in a separate avidity study conducted over a 30-day period. Horizontal bars represent means and SD for 13 replicates.

Bottom 2 figures are based on the 9 samples shown in the figures above; trendline represents a simple linear regression.

Supplementary Files

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