Anti-Pfs25 Human Plasma Reduces Transmission of Diverse Plasmodium falciparum Isolates by Direct Membrane Feeding Assays

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Running Title: TRA of Anti-Pfs25 Human Plasma against Field Isolates
25 ABBREVIATION:

26 TBV, transmission blocking vaccine
27 TBA, transmission blocking activity, referring to reduction of mosquito infection prevalence
28 TRA, transmission reducing activity, referring to reduction of oocyst density in infected mosquitoes
30 SMFA, standard membrane feeding assay
31 DMFA, direct membrane feeding assay
32 ELISA, enzyme-linked immunosorbent assay
ABSTRACT: Pfs25 is a leading candidate for a malaria transmission blocking vaccine whose potential has been demonstrated in a Phase 1 trial with recombinant Pfs25 formulated with Montanide® ISA51. Because of limited sequence polymorphism, the anti-Pfs25 antibodies induced by this vaccine are likely to have transmission blocking or reducing activity against most, if not all, field isolates. To test this hypothesis, we evaluated transmission blocking activities by membrane feeding assay of anti-Pfs25 plasma from the Pfs25/ISA51 Phase I trial, against *P. falciparum* parasites from patients in two different geographical regions of the world, Thailand and Burkina Faso. In parallel, parasite isolates from these patients were sequenced for Pfs25 gene and genotyped for seven microsatellites. The results indicate that despite different genetic backgrounds among parasites isolates, the Pfs25 sequences are highly conserved, with a single non-synonymous nucleotide polymorphism detected in one of 41 patients in Thailand and Burkina Faso. The anti-Pfs25 immune plasma had significantly higher transmission reducing activity against parasite isolates from the two geographic regions than the non-immune controls (P<0.0001).
INTRODUCTION

Despite decades of effort battling malaria, the disease is still a major cause of morbidity and mortality, mainly due to *Plasmodium falciparum*. Transmission-blocking vaccines (TBVs) that target sexual stage parasite development within the mosquito midgut are an integral part of the malaria control and elimination plan currently under development (1, 2). Among multiple TBV targets, Pfs25, an antigen expressed on the surface of *P. falciparum* zygotes and ookinetes in mosquito midguts, is a leading vaccine candidate with substantial evidence of induction of transmission blocking or reducing activities. A Phase 1 trial conducted on US adults testing a recombinant Pfs25 formulated with Montanide® ISA51 demonstrated transmission reducing activities in a Standard Membrane Feeding Assay (SMFA) using gametocyte cultures from the NF54 *P. falciparum* isolate (3).

While Pfs25 gene transcripts were detected in the blood stage gametocytes in the human host, the bulk of the Pfs25 expression commences only after fertilization in the mosquito host (4, 5). Due to little or no expression of the Pfs25 protein in the human host, it is unlikely to be naturally targeted by adaptive immunity. This is consistent with the absence of detectable anti-Pfs25 antibodies in human sera from highly endemic areas (6, 7), suggesting that the protein is not subject to selective pressure to evade the human immune system by antigenic variation and that the Pfs25 gene is likely highly conserved. Indeed, a survey of Pfs25 gene sequences from 9 parasite isolates from various geographic locations and 20 patients in Papua New Guinea revealed only one non-synonymous mutation (8, 9). Recent large-scale analysis of *P. falciparum* diversity in natural infections by deep sequencing showed only one synonymous mutation in the Pfs25 gene (25). It is therefore hypothesized that a recombinant Pfs25 vaccine based on the Pfs25 sequence in the *P. falciparum* clone 3D7 of isolate NF54 will induce strain-transcending
immunity against field isolates. In this study we evaluated the transmission blocking and reducing activity of human plasma from the Pfs25/ Montanide® ISA51 trial by a Direct Membrane Feeding Assay (DMFA). The assay uses \textit{P. falciparum} gametocyte-containing blood obtained from malaria patients from two distant endemic countries, Thailand and Burkina Faso, in two distinct local mosquito vectors, \textit{A. dirus} and \textit{A. gambiae}, respectively. Our results showed that the anti-Pfs25 human plasma reduces transmission of \textit{P. falciparum} isolates with diverse genetic background.

**MATERIALS AND METHOD**

**Processing of plasma.** The anti-Pfs25 plasma used in this study was collected by plasmapheresis of a volunteer from the Phase 1 trial of Pfs25/Montanide ISA51, under NIAID IRB approved protocol # 05-I-0118 (3). Plasma from a naïve volunteer (Interstate Blood Bank) was used as a control. Both anti-Pfs25 and naïve plasma were heat-inactivated prior to processing. In order to avoid clotting due to ABO blood type mismatch, we pre-adsorbed the plasma with whole AB\textsuperscript{+} type blood (Interstate Blood Bank Memphis, TN) to remove anti-A and anti-B antibodies, as described previously (10). Briefly, the whole AB\textsuperscript{+} type blood was washed to remove white cells and platelets. The AB\textsuperscript{+} red blood cell pellet was mixed at room temperature with the anti-Pfs25 immune plasma or the naïve-control plasma by gentle rotation for 20 min, and was then centrifuged at 2500 g for 10 min at 4°C. Supernatant was collected and filtered through a 0.22µ filter, aliquoted and stored at -80°C for feeding assays. Using a standard enzyme-linked immunosorbent assay (ELISA) as previously described (3), the anti-Pfs25 titer of this plasma was set to 3200 ELISA units. This plasma had been tested in standard membrane feeding assay (SMFA) in US and had demonstrated 68-83\% transmission reducing activity (TRA) (3).
DMFA. The assays were conducted separately at laboratories in Thailand and Burkina Faso.

Each laboratory used their own local laboratory reared mosquitoes exposed to gametocyte-infected blood from malaria patients in Burkina Faso and Thailand. The gametocyte-infected blood from malaria patients were collected under protocols approved by the institutional ethical committee in Burkina Faso (003-2009/CE-CM) or from the Ministry of Public Health in Thailand (Under Protocol # WRAIR 1308). Prior to testing the immune plasma at the two sites, the standard operating protocols (SOP) used by both laboratories were reviewed and harmonized to minimize assay variation. The only differences in the SOPs used by the two sites, in addition to the source of gametocytes, were the mosquito species, the volume and the hematocrit of the feeding mixture in the feeder, and the types of membrane used for feeding.

In Thailand, infections were carried out using a colony of the local vector Anopheles dirus and gametocyte carriers were detected among adult patients of the Malaria Clinic, Mae Sot, Tak Province. Gametocyte density in donor carriers was determined by a microscopy read of 500 leucocytes on thick blood smear. In Burkina Faso, an Anopheles gambiae (M molecular form) colony, established from the local natural vector population, was used. P. falciparum gametocyte carriers were selected among children screened in villages around Bobo Dioulasso. Gametocyte density in donor carriers was determined by a microscopy read of 1000 leucocytes on thick blood smear.

The gametocyte-infected blood was collected by venous blood draw and washed with incomplete RPMI. The blood was mixed with aliquots of anti-Pfs25 or heat-inactivated naïve control plasma. Taking into account the usual observed hematocrit in patients from the two study sites, the blood cell to plasma mixing ratio was 150 μL:180 μL in Thailand and 175 μL:180 μL in Burkina Faso. The mixture was incubated at room temperature for 15 minutes, and transferred in
glass feeders covered with Parafilm (in Burkina Faso) or Baudruche membrane (in Thailand) and
maintained at 37°C using a water jacket circulation system. Three feeders were used per blood
donor/plasma combination in order to avoid a potential feeder effect. Fifty 2-3 day old female
*An. gambiae* or one hundred 5-7 day old *An. dirus* mosquitoes were placed in each membrane
feeder and were allowed to feed for 30 minutes, after which all unengorged mosquitoes were
removed. Mosquitoes were provided a 10% sucrose solution daily until they were dissected for
oocyst counting at seven days (for *An. Gambiae*) or 9-11 days (for *An. Dirus*) after blood
feeding.

**Transmission blocking and reducing activity of the anti-Pfs25 plasma.** We considered two
types of activity of the anti-Pfs25 plasma, transmission blocking activity (TBA) and TRA. The
TBA measures the blocking of infection prevalence, and is calculated as: TBA=100*(1-
prevalence of infection for mosquitoes fed with immune plasma/ prevalence of infection for
control group). The TRA measures the reduction of oocyst density in infected mosquitoes, and
is calculated as: TRA=100* (1 - mean oocyst number with immune plasma/ mean oocyst number
in control group). We tested the TBA and TRA of the anti-Pfs25 plasma against parasite
isolates from 5 gametocyte carriers from Thailand, and 7 from Burkina Faso.

**Statistical methods.** To evaluate whether we used comparable parasite exposure for mosquito
vectors in Thailand and Burkina Faso, we used a Wilcoxon-Mann-Whitney (WMW) test for
testing gametocyte density between countries, and the t-distribution on the log transformed
values for confidence intervals on the geometric mean. To compare gametocyte density to mean
oocyst count, proportion of infected mosquitoes, or TRA, we used Spearman correlations with
confidence intervals calculated using rank transform methods.
We modeled the log of the mean oocyst count (for TRA) or the log of the infection prevalence (for TBA) as normally distributed. This assumption is motivated by the central limit theorem, which states that because each statistic comes from a mean (either the mean of the oocyst counts, or the mean of the indicators of infection) the normality assumption is reasonable even if the individual mosquito oocyst counts follow a more complicated model such the zero-truncated negative binomial model (11). The log of the mean can be treated as normally distributed using the delta method (12). For a single donor, we estimated the log of the ratio (test over control) of the oocyst means (for TRA) or prevalences (for TBA), using Welch’s two-sample t-test and the associated confidence intervals. In this case, the observations are the log means for the 6 feeders (3 with tested sera and 3 for control). This method automatically accounts for the feeder-to-feeder variability. The estimates and confidence limits are then transformed to TBA or TRA by the formula: $100(1-\exp(\log R))$, where $\log R$ is the estimate (or confidence limit) of the log ratio from the t-test. For testing the country effects we used meta-regression methods (13, 14). Meta-regression methods are typically used for meta-analyses where there are systematic effects of interest between studies. Here each donor acts like a study and the systematic effect is the country effect. We used the mean and variance of the logR effect for each donor from the t-test methods just described, and allow for donor variability through a random donor effect. We estimated the ratio of the effect from Burkina Faso over the effect from Thailand, where the effect is the test over control ratio for each country (estimated by the exponential of the average logR effect per country). Thus, confidence intervals on that ratio account for both the feeder variability and the donor variability.

**Pfs25 sequencing and microsatellite genotyping.** Blood from each gametocyte carrier was spotted on filter paper, dried, wrapped in aluminum foil and stored at ambient temperature.
Blood samples from an additional 29 *P. falciparum* carriers in Burkina Faso were added for Pfs25 sequencing. Genomic DNA was extracted from each gametocyte carrier blood sample using CTAB extraction buffer (15). Pfs25 gene was amplified by using forward PCR primer PF1 5’-CTTTGTTTCTTCAATTTATTC-3’ and reverse primer PR1 5’-TCATGGTATTTTTTTGTC-3’ upstream of the Pfs25 gene start codon and downstream of the Pfs25 stop codon, respectively. Both strands were sequenced using the Bigdye terminator v3.1 cycle sequencing kit (Applied Biosystems) and an Applied Biosystems sequencer. Sequences were assembled and verified using SeqScape (Applied Biosystems). The Pfs25 sequence from the NF54 *P. falciparum* isolate (8) was used as a reference sequence.

Microsatellites were genotyped for *P. falciparum* in blood from each gametocyte carrier using fluorescent-labeled forward primers. The markers used in this study (B5M5, C14M17, C13M63, B5M124, BM17, C1M67 and C1M4) were described previously (16, 17). Fragment analyses were conducted with a sequencer (Applied Biosystem) and alleles were sized relatively to an internal size standard using GENEMAPPER 4.0 (Applied Biosystem).

**RESULTS**

**Transmission blocking and reducing activities of the anti-Pfs25 plasma on *P. falciparum* gametocyte isolates in Thailand and Burkina Faso**

The DMFA were conducted by feeding vector mosquitoes on blood from *P. falciparum* gametocyte carriers. The geometric mean of gametocyte density of carriers in Burkina Faso was lower (133/µL (95% CI 78-227/µL)) than that of Thai carriers (409/µL (95% CI 62-2706/µL)),...
although there was no significant difference between the 2 countries (p=0.146 by WMW test).

To evaluate the effect of gametocyte density on baseline mosquito infectivity, we calculated the Spearman correlation ($r_s$) between gametocyte density and the proportion of infected mosquitoes or the mean oocyst density in mosquitoes from feeding cups using a control plasma. We found no significant correlation between proportion of infected mosquitoes and gametocyte density in the blood meal ($r_s$ = 0.46, 95% CI -0.16, 0.82, p=0.137), but there appears to be a strong positive correlation between the mean oocyst density in mosquitoes and gametocyte density ($r_s$ = 0.88, 95% CI 0.62, 0.97, p=0.0002).

We observed significant reductions in oocyst density in 10 of 12 feeding assays for mosquitoes fed with gametocyte containing blood mixed with anti-Pfs25 plasma compared to mosquitoes fed on the same infectious blood mixed with control sera (Table 1, Figure 1). The gametocyte density in the blood meal, however, did not strongly impact the TRA ($r_s$ = -0.18, 95% CI -0.68, 0.44, p=0.58), as the mean oocyst density in the controls was used to standardize the TRA. The activity may also be measured by TBA: inhibition of mosquito infection (prevalence). We observed a significant correlation between TRA and TBA by anti-Pfs25 plasma ($r_s$ = 0.76, 95% CI 0.32, 0.93, p=0.0045). However, there were fewer significant TBA events, with only 7 of 12 cases significantly blocking infection (Table 1, Figure 2). This lack of significance may be due to the high oocyst density in the infection control typically observed in these assays (18, Fig 2).

Meta-regression analysis was conducted to determine whether TBA and TRA, as measured by inhibition of infection prevalence and oocyst density, respectively, conducted in Thailand and Burkina Faso are comparable. The average TBA was 51.2% (95% CI 15.8, 71.8) for Thailand, and 31.0% (95% CI -9.4, 56.5) for Burkina Faso, whereas the average TRA was estimated as 86.1% (95% CI 68.6, 93.8) for Thailand, and 80.3% (95% CI 64.1, 89.2) for Burkina Faso.
Percent inhibition was calculated as $100\% (1-R)$, with $R$ being the ratio of test to control. The ratio of the ratios (RR) was used to compare the two countries $(RR = R(\text{Burkina Faso})/R(\text{Thailand}))$. A value of RR close to 1 would mean that the two countries have comparable effects. We estimate that $R_{\text{TBA}}=1.41$ (95% CI 0.69, 2.89), and $R_{\text{TRA}}=1.41$ (95% CI 0.51, 3.88). Despite the higher-than-one values in both $R_{\text{TBA}}$ and $R_{\text{TRA}}$, we find no significant differences between the two sites, and the confidence intervals of $R_{\text{TBA}}$ and $R_{\text{TRA}}$ rule out extreme differences.

The meta-regression methodology allows us to adjust for effects of gametocyte density on RRs by adding a log gametocyte density term to the linear meta-regression model. The adjusted $R_{\text{TRA}}$ ($R_{\text{TRA}}[\text{adj}]=1.87$, 95% CI 0.57, 6.13, $p=0.26$) shows no significant country effect, whereas the gametocyte adjusted $R_{\text{TBA}}$ ($R_{\text{TBA}}[\text{adj}]=2.21$, 95% CI 1.16, 4.20, $p=0.021$) does show a significant country effect, indicating DMFA parameters other than gametocyte density may contribute to the TBA readout.

**Pfs25 protein is highly conserved among field isolates with independent genetic background.**

The recombinant Pfs25 in the Pfs25/ISA51 vaccine was manufactured based on the sequence of isolate NF54 of the *P. falciparum* clone 3D7. We genotyped parasites from gametocyte carriers involved in the study by using 7 microsatellite markers, and sequenced the Pfs25 genes in these parasites and additional samples.

Genotyping was conducted using DNA from human blood samples containing both sexual and asexual blood stage parasites. The number of alleles detected by microsatellite markers allowed us to estimate the genetic diversity among the parasite population in the patients. Parasite
isolates from 4 of 5 Thai revealed only one allele for each of the 7 microsatellite markers, whereas 2 alleles were detected for one microsatellite in parasites from the fifth carrier (see Table 2). The microsatellite haplotypes identified in the individual Thai carriers were different indicated that while carriers from Thailand were infected with one or two parasite, each was infected with a different isolate. In Burkina Faso, however, parasite genetic diversity was much greater. The 7 Burkina Faso gametocyte carriers showed evidence of multiple infections with 2 to 9 different haplotypes detected per donor with at least 42 distinct genotypes or clones were present within this group (Table 2).

Despite the highly divergent genetic background of parasites among the carriers from the 2 countries, Pfs25 sequences revealed a very low level of diversity. Among the Thai isolates, one non-synonymous mutation was observed, compared to the previously published NF54 isolate (8). This mutation observed in the blood donor identified as Thai 3 has previously been detected in other geographical areas (23), results in a Glycine to Alanine substitution. No synonymous polymorphisms were detected among the Thai isolates. In Burkina Faso, no polymorphism, synonymous or non-synonymous, compared to the NF54 isolate was detected in the Pfs25 gene among all 7 gametocyte carriers and 29 additional parasite carriers.

Interestingly, the Pfs25 gene sequence of the donor with the lowest TBA (Thai 4) was identical to that in NF54. Conversely the anti-sera of the donor with the Pfs25 gene Alanine substitution (Thai 3) had highly significant TBA ($P_{TBA}=0.003$ and $P_{TBA}=0.019$). These results from our study suggest that sequence polymorphism may have little impact on efficacy of recombinant Pfs25 vaccines based on sequences in NF54 isolate.
DISCUSSION

Previously Arakawa and colleagues demonstrated effective transmission blocking activity against field isolates using anti-Pfs25 sera raised in rodents (19). Similarly in a pilot study we observed that pooled rabbit sera from animals immunized with Pfs25/Montanide ISA51 inhibited parasites from a Thai malaria patient with a single Pfs25 haplotype identical to the 3D7 strain (data not shown). This paper is the first report that human anti-Pfs25 sera can confer transmission blocking activity in a DMFA against field parasites responsible for local transmission. It supports our hypothesis that Pfs25 vaccines based on NF54 isolate sequence can induce strain-transcending immunity. The limited polymorphism of Pfs25 may be central to strain-transcending immunity and may facilitate Pfs25-based transmission-blocking vaccine development. We recognize this proof-of-concept was based on a single human serum with relatively high anti-Pfs25 titer, and that the observation needs to be confirmed by immune sera from future vaccine trials.

To minimize assay variability between two sites we harmonized the DMFA SOPs for blood processing, feeder set up, feed duration and temperature while gametocyte carrier recruitment (patients in Thai clinics versus asymptomatic Burkina Faso volunteers), hematocrit in blood meal, and mosquito species, were adapt to local conditions. Our results demonstrated similar anti-Pfs25 serum transmission reducing and/or blocking activity against local mosquito vectors in both sites.

We observed strong efficacy of the anti-Pfs25 plasma in reducing oocyst intensity (mean TRAs were above 80% in both Thailand and Burkina Faso) and a more limited blocking activity (mean TBAs were 31% and 51.2% in Thailand and Burkina Faso, respectively). The same plasma had
been tested in SMFA in US and had demonstrated 68-83% TRA (3). Further analyses of the
same data sets revealed lower TBA (0-11%), in multiple assays with mean oocyst densities of
5.1-52.1 in control mosquitoes fed with an autologous pre-immune serum [Wu Y, unpublished].
The TRA and TBA results by DMFAs in Burkina Faso and Thailand seem comparable to those
by SMFAs in US. We therefore observed similar efficacies of the anti Pfs25 plasma against
cultured and field parasites.

It was anticipated that the anti-Pfs25 plasma would show a lower effect on mosquito infection
prevalence (TBA) than on oocyst density (TRA) in these assays. As recently highlighted by
Churcher and colleagues, variation in oocyst density associated with little or no change in
prevalence occurs at high levels of infection, with high infection prevalence and high oocyst
density. This is due to the relationship between prevalence and oocyst density where infection
prevalence reaches a plateau close to 100% when oocyst density is high (18). Consequently,
TBA is more dependent on control infection intensity than TRA, and a higher TBA may be
observed when mosquitoes are fed on blood meals with lower infectious gametocyte densities.
In the present study, oocyst density in the control groups were high as defined by Churcher and
colleagues (18), probably as a result of selecting volunteer blood donors with higher gametocyte
densities, whereas in natural settings wild mosquito vectors may be infected by gametocyte
carriers at a sub microscopic detection level (24). Actual exposure of mosquitoes to P.
falciparum in natural settings remains poorly documented (20, 21). Studies to characterize the
parasite intensity in naturally infected malaria vectors and subsequent transmissibility to human
populations could potentially fill a major gap of knowledge for evaluating TBV candidates at
relevant infection levels.
ACKNOWLEDGEMENT

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REFERENCE


FIGURE LEGEND

Figure 1: Transmission Reduction Activity (TRA) measured as percent reduction on mean oocyst density. For each subject we give the estimate and the 95% confidence interval. The “Meta Analysis” columns represent the overall estimate from that country with the 95% confidence interval calculated by meta regression.

Figure 2: Transmission Blocking Activity (TBA) measured as percent reduction on infection prevalence. For each subject we give the estimate and the 95% confidence interval. The “Meta Analysis” columns represent the overall estimate from that country with the 95% confidence interval calculated by meta regression.
### Table 1. Transmission blocking and reducing activity in DMFAs conducted in Thailand and Burkina Faso

<table>
<thead>
<tr>
<th>Study Site</th>
<th>Gamocyte Carrier</th>
<th>No. Mosquitoes Dissected</th>
<th>% Reduction in Prevalence (95% Confidence Interval)</th>
<th>Ooc Density Mean; Median (IQR)</th>
<th>% Reduction in Oocyst Density</th>
<th>p-value</th>
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<tr>
<td><strong>Thailand</strong></td>
<td></td>
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<td>Thai 1</td>
<td>96/120</td>
<td>17/120</td>
<td>82.5 (72.1, 89.0)</td>
<td>20.1; 9 (1-30)</td>
<td>96.9 (88.9, 99.2)</td>
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<td>Thai 2</td>
<td>52/60</td>
<td>34/60</td>
<td>34.8 (14.5, 50.3)</td>
<td>144.1; 140 (96-236)</td>
<td>91.8 (66.5, 98.0)</td>
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<td>Thai 3</td>
<td>74/120</td>
<td>17/120</td>
<td>77.4 (59.2, 87.5)</td>
<td>2.6; 2 (0-4)</td>
<td>91.2 (56.9, 98.2)</td>
<td>0.0190</td>
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<tr>
<td>Thai 4</td>
<td>51/60</td>
<td>48/60</td>
<td>5.6 (-20.5, 26.0)</td>
<td>149.3; 112 (8-246)</td>
<td>33.8 (-67.1, 73.8)</td>
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<td>Thai 5</td>
<td>79/84</td>
<td>63/66</td>
<td>-1.1 (-15.1, 11.2)</td>
<td>222.9; 162 (49-345)</td>
<td>71.0 (-21.7, 93.1)</td>
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<tr>
<td><strong>Burkina Faso</strong></td>
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<td></td>
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<tr>
<td>BF 1</td>
<td>53/63</td>
<td>53/73</td>
<td>14.9 (-7.6, 32.7)</td>
<td>33.4; 28 (6-49)</td>
<td>80.6 (58.5, 91.0)</td>
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<td>BF 2</td>
<td>45/47</td>
<td>93/109</td>
<td>10.5 (-10.4, 27.5)</td>
<td>86.4; 88 (24-133)</td>
<td>73.6 (63.6, 80.9)</td>
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<td>BF 3</td>
<td>80/93</td>
<td>60/96</td>
<td>27.1 (5.2, 43.9)</td>
<td>13.7; 13 (3-23)</td>
<td>72.0 (63.9, 78.2)</td>
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<tr>
<td>BF 4</td>
<td>42/68</td>
<td>19/61</td>
<td>48.5 (1.2, 73.2)</td>
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<td>BF 5</td>
<td>64/82</td>
<td>67/104</td>
<td>19.2 (-10.4, 40.8)</td>
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<td>BF 6</td>
<td>60/75</td>
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<td>48.7 (31.9, 61.3)</td>
<td>11.1; 5 (1-14)</td>
<td>84.6 (66.2, 93.0)</td>
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<td>BF 7</td>
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<td>65/130</td>
<td>40.1 (25.8, 51.6)</td>
<td>14.0; 13 (2-21)</td>
<td>80.9 (72.4, 86.7)</td>
<td>0.0003</td>
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a The DMFAs were conducted using *A. dirus* and *A. gambiae* mosquitoes in Thailand and Burkina Faso, respectively.

b IQR, Inter Quartile Range is the difference between the third and the first quartiles.

c % reduction was calculated by comparing the infection prevalence or the oocyst count in mosquitoes fed with anti-Pfs25 test plasma and a non-immune control plasma after combing information from feeders using log transformations. For details on the estimation and calculation of the 95% confidence intervals and p-values see the statistical methods section.
Table 2. *P. falciparum* microsatellite allele sizes in blood samples from gametocyte carriers

<table>
<thead>
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<th>Study Site</th>
<th>Gametocyte Carrier</th>
<th>Microsatellite markers</th>
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<td>Thailand</td>
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<td>221</td>
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<td>172/171/211/220</td>
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<tr>
<td>Thai 2</td>
<td>226</td>
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<td>Thai 3</td>
<td>214</td>
<td>186</td>
<td>201/177</td>
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<tr>
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<td>ND</td>
<td>196</td>
<td>381/152</td>
</tr>
<tr>
<td>Thai 5</td>
<td>230</td>
<td>167/163</td>
<td>240/171</td>
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<tr>
<td>Burkina Faso</td>
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<tr>
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<td>211/217/230</td>
<td>205/215/226</td>
<td>232/247</td>
</tr>
<tr>
<td>BF 4</td>
<td>198/204/217</td>
<td>178/186/190</td>
<td>199/203/216</td>
</tr>
<tr>
<td>BF 5</td>
<td>207/224</td>
<td>228</td>
<td>219/200/211</td>
</tr>
<tr>
<td>BF 6</td>
<td>211/220/224</td>
<td>180/192</td>
<td>219/200/211</td>
</tr>
<tr>
<td>BF 7</td>
<td>192/204/211</td>
<td>180/192/207</td>
<td>177/183/188/194</td>
</tr>
</tbody>
</table>

a Number of detected parasite haplotypes in the human blood donor, according to the maximal number of alleles observed

b Not detected
Figure 1

Percent Inhibition (mean oocyst count)

Thai 1
Thai 2
Thai 3
Thai 4
Thai 5
Meta Analysis
BF 1
BF 2
BF 3
BF 4
BF 5
BF 6
BF 7
Meta Analysis
Figure 2