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Anti-malarial Antibody Responses & Applications for Assessing Malaria Exposure

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Anti-malarial Antibody Responses & Applications for Assessing Malaria Exposure

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ABSTRACT

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This dissertation describes the discovery of highly informative serologic biomarkers of recent exposure to *Plasmodium falciparum,* the most deadly species causing malaria. An innovative approach that combined detailed individual-level exposure data, high-throughput screening of hundreds of antibody responses, and robust statistical methods was used to identify the most informative signatures of exposure. The novel antigens described here and, more importantly, the outlined approach for biomarker discovery will allow for the development of public health and research tools that are imperative for the control and elimination of malaria. Additionally, the methodologies outlined here are highly applicable to the discovery of biomarkers of exposure for other infectious diseases.

Serology has been used for decades to measure exposure to *Plasmodium* species and other infectious diseases. While useful, most existing assays for measuring *P. falciparum* exposure have been based on population-level responses to a few target antigens chosen by convenience rather than utility, resulting in relatively coarse exposure estimates. Here, detailed assessments of malaria exposure in Malian and Ugandan children were used to identify novel serologic biomarkers of malaria exposure and calibrate responses to quantitative estimates of individual exposure. The power of obtaining these individual-level estimates was illustrated by their ability to accurately identify individuals with infection in the recent past; to obtain precise estimates of malaria incidence in a population from cross-sectional samples of as few as 20 individuals; and to accurately estimate heterogeneity in recent exposure within a community using data from a single time point.

Interest in the development of improved serologic assays has increased in recent years as more investment is made in malaria control and elimination. There is need for widely available, accurate estimates of malaria exposure that will allow for targeting and evaluation of public health interventions. This dissertation initiates a response to the call to develop accurate field-based assays for rapid and cost-effective assessment of malaria exposure, with the ultimate goal of putting cohort-quality data into the hands of malaria control programs.

DEDICATION

This dissertation is dedicated to my parents who have offered unwavering support through my academic and personal endeavors. Thanks Mom and Dad for always believing in me and for encouraging me to strive for my dreams. I would also like to thank Nonna, Zia, and Kristina.

You do not always understand what my journey is or why I am doing it, but still love and unconditionally support me anyway.

On Children

Kahlil Gibran

Your children are not your children.
They are the sons and daughters of Life's longing for itself.
They come through you but not from you,
And though they are with you yet they belong not to you.

You may give them your love but not your thoughts,
For they have their own thoughts.
You may house their bodies but not their souls,
For their souls dwell in the house of tomorrow,
which you cannot visit, not even in your dreams.
You may strive to be like them,
but seek not to make them like you.
For life goes not backward nor tarries with yesterday.

You are the bows from which your children
as living arrows are sent forth.
The archer sees the mark upon the path of the infinite,
and He bends you with His might
that His arrows may go swift and far.
Let your bending in the archer's hand be for gladness;
For even as He loves the arrow that flies,
so He loves also the bow that is stable.

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INTRODUCTION

BACKGROUND

Half of the global population inhabits areas where there is a risk of contracting malaria. The World Health Organization estimates that there were over 200 million clinical episodes of disease and approximately 627,000 deaths due to malaria in 2012 (1). Nearly 90% of deaths occur in children under the age of five who reside in sub-Saharan Africa (2–4), where malaria kills one child every minute (5). In addition to the dramatic morbidity and mortality associated with malaria, the disease exerts an economic toll on endemic countries (6, 7). Malaria leads to the annual loss of approximately $400 billion USD in African countries, further impeding their economic development (7). Malaria clearly remains one of the most important infectious disease problems in the world (1, 3, 8).

Malaria is a mosquito-borne disease caused by intracellular eukaryotic protists of the genus *Plasmodium.* Of the five *Plasmodium* species that are responsible for causing malaria in humans, *P. falciparum* causes the most severe disease and is responsible for the majority of malaria deaths globally (9). Found worldwide in tropical and subtropical areas, *P. falciparum* is the most prevalent malaria species in Africa. Infected female *Anopheles* mosquitoes inoculate *P. falciparum* sporozoites into the human host during blood meals. The sporozoites then enter liver cells, where they mature and replicate. After this initial replication in the liver, the parasites infect and replicate asexually inside of red blood cells. Some parasites differentiate into sexual gametocytes, which are ingested by an *Anopheles* during a blood meal and recombine within the mosquito midgut. The resulting oocysts release sporozoites, which travel to the mosquito's salivary glands to complete the malaria life cycle (Figure 1, (10)).

Immunity to malaria develops gradually with years of repeated *Plasmodium* infections (11). Partial malaria immunity, in which there is a reduction in clinical presentation of the disease in the presence of *Plasmodium* infection without complete parasite clearance, develops more quickly in children with higher exposure to malaria parasites (12). In areas with moderate to high malaria transmission, protection from severe disease is acquired in young children (usually by 2-5 years of age), and the rate of symptomatic illness decreases in early adolescence (13). Anti-parasite immunity generally increases with the maturation of the immune system. Parasite densities following infection in adults frequently remain at low levels undetectable by microscopy (14). Asymptomatic infections, generally defined by blood-stage malaria infections that do not induce fever or other symptoms that would cause the individual to seek treatment, can persist for months (15, 16). Asymptomatically infected individuals have been reported to have lower asexual but higher gametocyte densities than symptomatic persons (17) and are therefore more infectious. Thus, it is important to keep in mind that individuals with both symptomatic and asymptomatic infections contribute to the cycle of malaria transmission.

Accurate Measurement of Exposure Is Essential for Malaria Control and Elimination

In the last several years, a number of organizations have spearheaded a global movement to combat malaria. Following assertions at the Gates Malaria Forum in October 2007, the World Health Organization, the Board of the Roll Back Malaria Partnership, and many other institutions have supported a paradigm shift from malaria control to eradication, defined as reducing the worldwide incidence of malaria to zero permanently (18). In 2011, the UN Secretary-General set a target of zero malaria deaths by the year 2015 (19). Funding for malaria increased from $149 million USD in 2000 to almost $1.2 billion in 2008 (20, 21), and lead to the rapid expansion of malaria control interventions in Africa (22, 23).

In higher transmission areas, malaria control is of primary importance. Accurately estimating local exposure to malaria parasites, defined as the rate at which people are infected by mosquitoes carrying *Plasmodium* species in a given area, is essential for assessing the extent of disease burden in an area, planning evidence-based control strategies, and evaluating the impact of interventions over time (24–27). For control, it is necessary to make repeated measures of parasite exposure over time in a particular location in order to appropriately target and assess control interventions and evaluate the progress made towards reducing the burden of malaria (28, 29). Longitudinal monitoring of deviations in parasite exposure over time would allow malaria control programs to adapt control strategies to changing local conditions (30).

With the scaling up of interventions for prevention, diagnosis, and treatment, the malaria burden has declined in sub-Saharan Africa. As malaria transmission declines, the possibility of elimination, defined as the termination of local transmission of malaria within a defined geographical region, is increasingly being considered (31, 32). On the path to the elimination of malaria, it is imperative to be able to detect the highest possible fraction of *Plasmodium* infections in the general population to interrupt transmission. Especially in areas with lower malaria burdens, where a higher percentage of infections are asymptomatic, it is important to actively identify *Plasmodium* infections rather than relying on passive detection of cases presenting to health facilities because individuals with both symptomatic malaria and asymptomatic infections can transmit parasites onwards (32). Furthermore, particularly in low-transmission areas, microscopy can miss a substantial proportion of *P. falciparum* infections (33). Studies have demonstrated that submicroscopic infections are important contributors to infection of mosquitos in areas with low or very low transmission intensity (34); this important reservoir sustaining malaria transmission must also be considered for effective control. In areas that have eliminated malaria, long-term active monitoring will need to be continued until eradication is achieved and diagnostics are no longer required (32).

Malaria parasite exposure is heterogeneous, with approximately 80% of transmission occurring within 20% of the population (25, 35–40). Variation is apparent at nearly every spatial scale (41), and has been attributed to multiple factors such as the variable ecology of local vectors of transmission, local patterns of human host and vector contact, and intrinsic factors of the human host (42–44). To be effective, therefore, malaria control interventions need to target the areas of highest risk, whether

these are districts within in a country or, especially in areas of low exposure, hotspots within a village (45–48). With approximately $2 billion USD spent on global malaria control each year, there is an obligation not only to target these resources appropriately, but also to evaluate their effectiveness (49, 50). Unfortunately, insufficient data are available to guide the deployment of costly malaria interventions or measure their impact in real world settings. There is currently no consensus on which methods are most appropriate for programmatic monitoring of malaria endemicity (disease intensity) over time. Accurate, inexpensive methods for measuring exposure to malaria parasites would greatly improve the ability of policy-makers to determine which interventions are working and to decide where new or modified interventions are needed.

Current Methods for Measuring Exposure to Malaria Parasites Are Too Expensive and/or Inaccurate

Malaria exposure varies with location in a manner that is predictable on a large scale from climatic and geographical data (51). Although considerable progress has been made in large scale mapping of malaria risk across Africa using data on the endemic level of malaria in specific locations (52–55), these maps have poor predictive capacity at the level of individual communities, villages, and neighborhoods (56). Furthermore, these predictions are unresponsive to short term changes such as population migration, seasonal or periodic climate change, or malaria control interventions. To achieve this degree of spatial and temporal detail, repeated evaluations of transmission intensity from the location of interest are necessary (52). These data are imperative to identify foci of residual or reintroduced infection and locations where the incidence of malaria is low enough to justify the transition from endpoints of malaria control to elimination (32).

Transmission intensity, which can be defined as the number of times per day that a *Plasmodium* infection is initiated in a human or alternatively as the number of times that the pathogen becomes established within a mosquito, is dependent on a number of factors such as the efficiency of local mosquito populations at spreading the parasite, human immunity, and public health interventions in place (57, 58). Different methods for measuring malaria transmission intensity evaluate the burden of malaria parasites in a community at various stages of the life cycle, e.g. mosquito to human transmission, blood stage infection in humans, and human to mosquito transmission (59). However, all of these methods ultimately attempt to quantify the risk of human infection. As used here, the term “exposure” refers assessments made by any of the metrics described below.

The Entomological Inoculation Rate (EIR) estimates the number of infectious bites received per person per unit time, and is the most direct indicator of, and is widely considered the gold standard for, measuring malaria infections passed from mosquito to human (60). The EIR is the product of the number of mosquito bites per person per year and the proportion of mosquitos with sporozoites in their salivary glands (59). Measuring the EIR requires estimating the number of mosquitoes biting per night, and human landing catches, pyrethroid spray catches, exit traps, and CDC light traps have all be employed to catch and count the number of mosquitos attempting to feed on a human.

The captured mosquitos must then be examined for the presence of sporozoites. These measurements entail serious challenges in logistics, interpretation, and costs (59, 61, 62). Different trapping methods bias the number and types of mosquitoes captured (63, 64). While human landing catches are considered to be the best method for mosquito capture, this method ignores bednet use and other important human behaviors that make the EIR difficult to interpret, and there are ethical issues with putting the individuals conducting catches at risk of malaria infection (64). The sporozoite rate can be estimated through dissection of mosquito salivary glands, or with ELISAs or PCR. The validity of EIR estimates can be affected by false positive results in ELISAs or PCR, or by subjectivity in evaluations by dissection. Furthermore, measurements of the EIR frequently lack the precision necessary to detect spatial variation or changes in exposure over time (39, 59, 65, 66). Sporozoite infection rates are low even in highly endemic areas, and mosquito distributions can vary widely over short distances within a single settlement (67). Moreover, in areas of low exposure, numbers of captured infected mosquitoes are commonly too small to generate meaningful results. Due to the short life span of the mosquito vector, estimates based on the EIR require frequent and large scale re-sampling. The expense and logistical challenges of assessing the EIR causes these estimates of malaria exposure to be performed infrequently in most endemic areas.

Parasite prevalence or parasite rate (PR), on the other hand, is relatively inexpensive to perform. PR is simply a measurement of the proportion of people in a representative sample from the population, usually taken from a cross-sectional survey, who have parasites detectable in their blood (by microscopy, rapid diagnostic tests, or PCR). PR can also be calculated from routine health facility data. Because it is simple and inexpensive, PR is the most frequently used metric, and thus forms the basis of large-scale maps of malaria exposure (68, 69). Unfortunately, PR is a relatively inefficient measure of exposure. Accuracy of PR is biased by the distribution of parasite densities in a population, which may vary by season, age, and exposure (59, 70, 71). Parasitemia can be short-lived, and periodic PR surveys may completely miss sporadic changes in exposure. Additionally, long term carriage of drug-resistant parasites after treatment can lead to overestimates of PR. Conversely, acquired immunity can cause exposure to be underestimated, especially in high exposure settings, as immune control of the densities of blood stage parasites renders them less easily detectable. Similarly, access to effective anti-malarials and treatment seeking behavior can affect estimates of PR, independent of intensity of exposure. Furthermore, accurate assessments of PR are strongly dependent on the sensitivity of the diagnostic test used. For these reasons, it is difficult to interpret PR measurements across sites (59). Furthermore, data from individual subjects do not provide meaningful quantitative information since each person can only be either infected or uninfected. Where PR is low, large sample sizes are needed to obtain a single point estimate of exposure, and PR saturates at high exposure making it difficult to evaluate changes in exposure (70, 72). Due to the variable duration of individual infections, resampling is required to gain an accurate view of the overall malaria burden. However, assessments of PR are not performed frequently enough to avoid sampling errors caused by short-term seasonal changes or to identify long term trends in exposure.

The Incidence of Symptomatic Malaria, the rate at which new cases of clinical malaria (fever and parasitemia) occur in the population, directly estimates the burden of disease in that population. Incidence can be passively estimated from routine hospital surveillance records, which are notoriously unreliable. Estimation of malaria incidence through passive case detection assumes that every health facility reports data accurately, every incident infection has access to and presents at a health facility, and that temporal coverage is complete. Health centers often presumptively diagnose individuals with symptoms of malaria, overestimating the number of cases (73). Additionally, some studies have found that fewer than 20% of malaria cases are reported to official health monitoring systems (30, 73, 74). Alternatively, incidence can be more accurately assessed from active case detection in cohort studies after artificially clearing participants of infection. More incident malaria cases are captured through active case detection (75). However, in areas of low exposure, large sample sizes are required for precision. Additionally, cohort studies are expensive and time consuming and are not scalable (76, 77). In either case, disease incidence is reduced by acquired immunity and thus may not accurately reflect exposure (78, 79). Additionally, at high exposure levels, incidence saturates and estimates do not exceed those observed in areas with intermediate exposure (78, 79). Thus, incidence measures will not be able to detect declining exposure until there is a significant reduction in cases (80, 81).

The Force of Infection (FOI) estimates the number of infections per person per unit time using cohort studies or repeat cross-sectional surveys. Current infections and all incident human malaria infections, whether symptomatic or asymptomatic, occurring within a certain period of time are included in the FOI (59). The FOI is appealing for a number of reasons: infection rates have direct meaning for mathematical models, epidemiological and immunological studies, and public health; and measurements in individual people over time allow for fine spatial and temporal resolution over a wide range of exposure. The FOI can be estimated by treating study participants with antimalarial drugs and monitoring the time it takes for them to become parasitemic, or by observing patterns of parasitemia over time (82–87). Natural fluctuations in parasite density and the occurrence of superinfection limit the accuracy of these measurements, especially when exposure is high, but newer methods have used molecular genotyping to identify the acquisition of new parasite clones in the blood over time (88–90). The molecular FOI, which takes the number of new parasite clones acquired into account, allows newly acquired infections to be measured in the presence of previously acquired infections and has been shown to have great utility in explaining the epidemiology of malaria by accurately estimating exposure (90–92). For these reasons, the FOI via molecular analysis of cohort data can be considered to be the gold standard for exposure. However, FOI estimates are affected by age, seasonality, insecticide-treated net use, and chemotherapy. Additionally, FOI accuracy is dependent on the sensitivity and specificity with which microscopy is able to detect infections (59). Similarly, molecular FOI estimates are dependent on the ability of PCR to detect all parasite clones. Finally, the expense required to follow cohorts prohibits this method from being widely used for malaria surveillance.

In order to evaluate advancements towards the goal of eradication, robust and highly sensitive methods for rapid assessment of changes in malaria exposure at the

level of specific individual communities must be developed (93). Current methods to directly measure malaria exposure within a community have significant limitations. The practical implication of a lack of reliable malaria exposure data is that the success or failure of control measures cannot be monitored carefully. Thus, new methods that can rapidly, sensitively, and reproducibly measure exposure are needed to carefully monitor public health interventions and progress towards the elimination of malaria (94).

Serological assessment of malaria exposure

The ideal tool for assessing malaria endemicity would be one that integrates malaria exposure over a period of time. An immunologic assay incorporating serological markers of infection (i.e., anti-malaria antibodies) would be a valuable tool for surveying changes in exposure intensity and for estimating the impact of control interventions (93). Antibody responses are known to provide protection from malaria (95, 96), although the specific antibodies that serve as correlates of protection are as yet undefined. Kinetics of antibody responses to specific malaria antigens have only been evaluated for a small subset of antigens, but have been shown to differ (97–99). Although the exact duration of anti-malaria immunity is debated (100), antibodies clearly persist markedly longer than parasite infections in the blood or the lifespan of infected mosquitos (101), making serological tools potentially more robust and sensitive than PR or EIR. Critically, serology gives accurate measures of exposure even with very low exposure, where infectious mosquitoes and infected individuals are rarely found in cross-sectional surveys (102). As the antibody response to *Plasmodium* is a function of the number and timing of prior infections, quantification of the serological response to malaria antigens reflects exposure over an extended period (102, 103). Thus, unlike estimates of PR or EIR, which require frequent resampling to track changes in malaria endemicity over time, a single measurement of the relatively long-lived serological response to malaria antigens should capture long-term trends in temporal and spatial variation in exposure (102, 104). Therefore, assessment of antibody responses to appropriately selected *Plasmodium* antigens should allow for estimating past exposure to malaria.

Prior to development of synthetic or recombinant antigens, immunofluorescence antibody tests (IFAT), which measured prevalence of antibodies to both the sporozoite and blood stages of the parasite (103, 105), were used to estimate malaria endemicity. These assays were never widely used and eventually fell into disfavor because of the need to culture parasites in vitro to prepare slides, dependence on expensive fluorescence microscopes, and because slide reading was highly subjective and difficult to standardize. Subsequently, enzyme-linked immunosorbent assays (ELISAs) against parasite antigens have been shown to be potentially useful epidemiological tools (106, 107).

The impacts of interventions that reduce malaria parasite exposure have been assessed in large-scale serological surveys. A WHO research project conducted in northern Nigeria from 1970 to 1975 demonstrated that antibody prevalence and levels mirror recent changes in malaria exposure (108). Anti-malarial antibody responses fell abruptly with the application of residual insecticide spraying and mass drug administration and rebounded after the intervention ended.

More recently, in north-east Tanzania, the prevalence of IgG antibodies against the *Plasmodium* blood-stage antigen MSP-1_{19} was highly associated with altitude, a proxy for exposure intensity (102). Although PR also showed significant correlations with altitude, this measure was subject to local and seasonal variation (109). The serological responses, by contrast, were highly correlated with recent estimates of EIR. This is probably because serology integrates cumulative individual exposure over time, circumventing problems associated with cross-sectional sampling and seasonal variation in exposure. Using these results, a technique for modeling average prior exposure based on the presence of an IgG response to *Plasmodium* antigens (as detected by ELISA) was developed. This technique involved calculating the MSP-1_{19} IgG seroconversion rate (SCR), i.e. the estimated rate at which individuals in a population develop anti-malarial antibodies, by measuring the prevalence of antibodies in different age groups (102). As the SCR takes malaria exposure over time into account, it allows temporal exposure patterns to be studied (59).

SCRs have been generated for several locations in Africa (110–112), Asia (113), and the Pacific (114). SCRs are highly correlated with several independent measures of malaria exposure intensity, such as incidence in young children and averaged PR and EIR values. Historical reductions in local malaria exposure intensity due to successful control strategies in Tanzania (111), Vanuatu (114), Equatorial Guinea (112), and Swaziland (115) can be revealed by significant reductions in SCRs among younger cohorts born after the implementation of the intervention. However, in a single study investigating the utility of the serology for reconstructing long-term malaria trends in a region of western Kenya with high malaria exposure (where considerable exposure continues even after intervention reduced exposure), the SCR was not useful for the retrospective analysis of these historical changes (24).

A limitation of current serologic assays and the SCR is that they look at the prevalence of response to only one or a few antigens, thereby requiring that curves be fitted to a fairly large number of subjects to get a population-level estimate of exposure. Additionally, the original SCR framework estimates a single exposure rate averaged over a period of years. This increases the expense of surveillance and may limit the ability to assess fine-scale spatial variation in exposure. Furthermore, as antibodies can persist for years after exposure, the SCR is not sensitive to short term changes in exposure intensity (59). Also, the SCR has not yet proven useful at detecting less than log-fold differences in exposure intensity (102). Another limitation is that current assays dichotomize the total IgG response as present or absent, ignoring potentially valuable information provided by the titer of the response already present in the readout of the ELISA assay. Antibody titers may have a greater ability to describe endemicity than antibody prevalence. Also, titers that decay quickly after exposure (98) have the potential to enable assessment of recent as opposed to long-term exposure. Tools that are more informative, for retrospective analyses in areas of high endemicity or for detection of very recent changes in exposure, may require the identification of alternative malaria antigens that induce responses that are more highly informative of past exposure.

Towards the development of serologic assays for estimating the population-level dynamics of malaria exposure

Although serological methods such as the ELISA have yet to be thoroughly standardized and validated in settings with different intensities of exposure (116), they offer the potential for rapid and accurate assessment of malaria exposure. These assays can be performed on filter paper blood spots (117), which are simple to collect in large numbers in the field, store, and transport. Additionally, ELISAs are sensitive, specific, high throughput and relatively easy to standardize within and between laboratories in resource-poor settings. As data from a single cross-sectional serological survey can, in theory, be used to generate a point estimate of the current malaria exposure intensity as well past exposure history, serologic assays are potentially useful from a programmatic monitoring standpoint. With increasing attention and funding being given to malaria control and elimination, there is a need for tools that can accurately measure the epidemiological impact of interventions over extended periods.

The ideal optimized serologic assay(s) for malaria surveillance for use in control programs would be able to provide information about recent and/or long-term exposure, and be highly sensitive and species specific (118). Future serologic assays should be able to provide information on dynamic changes in exposure so that rapid changes, such as those resulting from control interventions, can be quickly identified. Assays specifically tailored to assess recent as well as long-term exposure to malaria need to be developed. The information present in antibody titers and other potentially important aspects of the response, namely IgG subclass and affinity, need to be evaluated. As serologic assessments of exposure are less widely accepted than traditional measurements of EIR or PR, future studies must rigorously demonstrate the accuracy of serologic assays in multiple epidemiologic settings, directly comparing results of these assays to EIR and PR. This will also require that intended target populations for particular applications be clearly defined. Also, methodologies need to be standardized so that serologic data can be compared across different populations and regions (118).

Antibody responses to single antigens may have limited value as biomarkers of exposure, making it possible that several antigens will need to be included in serologic assays (118). Additionally, most serology data has been generated from ELISAs measuring responses to a small subset of antigens (mainly apical membrane antigen 1 (AMA1), merozoite surface protein 1 (MSP1), merozoite surface protein 2 (MSP2), and circumsporozoite protein (CSP)), but responses to other malaria antigens are likely to be of value. For areas of low exposure intensity, as a proxy for a test of the sensitivity of the assay, the proportion of individuals in Cambodia who were parasite positive who also had antibodies to either MSP1 or AMA1 accounted for nearly 90% of the individuals exposed to parasites (119). It may be that the judicious addition of one or more other antigens may increase this sensitivity, and by combining the antigens in a single assay we will be able to devise a very high sensitivity assay specifically for low exposure settings. On the other end of the spectrum, CSP (a protein secreted during the sporozoite stage of infection) has proven to be a useful antigen for high exposure sites. In areas where exposure to the parasite is very high, maximum seroprevalence for blood stage antigens (such as AMA1 and MSP1) plateaus at a relatively young age, making it difficult to accurately discern the rate at which the community becomes

seropositive. In an area of Uganda where the EIR is ≥1500 infective bites per person year, seroprevalence to the more immunogenic antigens, such as AMA1, saturated at very young ages, whereas responses to CSP increased gradually with age (120). Even though there are more than 5000 proteins expressed by *Plasmodium* species, very few have been examined in detail (121), especially in regards to their potential utility in assays for sero-surveillance. Broad assessment of candidate antigens needs to be undertaken to identify specific antibody responses that are most informative of malaria exposure.

The development and appropriate application of serologic biomarkers of malaria exposure has great potential to strengthen malaria control and elimination programs. In this project, we focused on identifying and characterizing novel antigens that induce antibody responses informative of prior malaria exposure in detailed cohorts from high exposure settings in Africa. Careful selection of specific serological markers for the intensity of malaria exposure should allow for the rapid and comparatively inexpensive development of detailed pictures of variations in malaria endemicity. As *P. falciparum* is the predominant species in the areas under study, we employed protein microarrays to comprehensively evaluate a large proportion of the *P. falciparum* protein repertoire for the induction of antibody responses highly associated with exposure. We evaluated novel statistical methodologies, which—by considering titers of multiple antibodies—potentially enable more precise estimates of exposure to be obtained from fewer subjects. This project represents a first—but key—step in the development of highly informative assays for sero-surveillance.

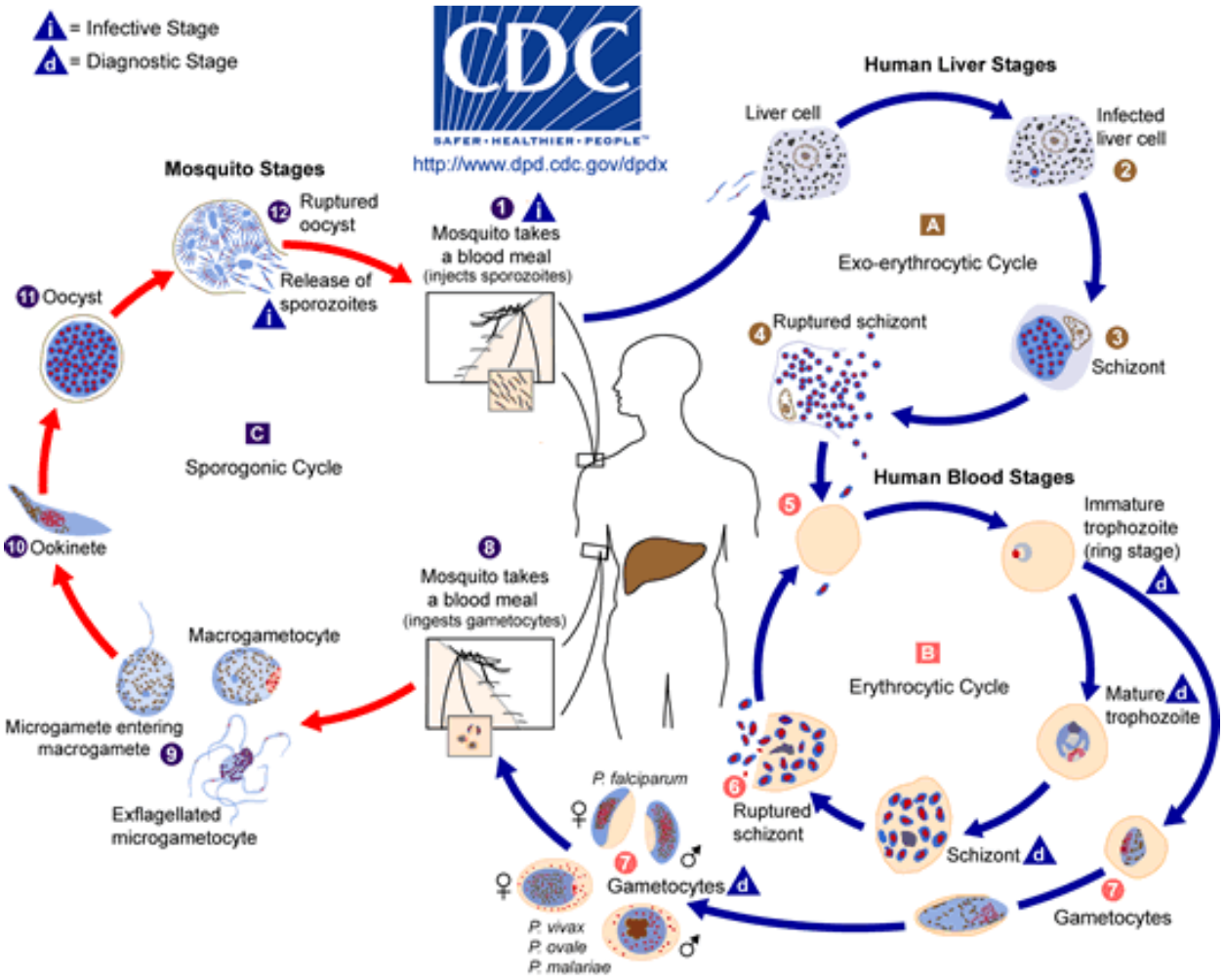

Figure 1. Life cycle of *P. falciparum* (10). Malaria infection in humans is initiated when sporozoites are injected with the saliva of a female Anophelene mosquito as she feeds. Sporozoites migrate to the liver, where they invade hepatocytes. Inside of hepatocytes, sporozoites divide into merozoites. The asexual blood-stage of the life cycle is intitiated when merozoites are released from the infected hepatocytes and invade erythrocytes. Inside of erythrocytes, *P. falciparum* develops through ring, trophozoite and schizont stages to produce multiple merozoites. The daughter merozoites released invade other erythrocytes to continue the asexual intraerythrocytic cycle, and this cycle of infection is responsible for the clinical manifestations of malaria. Some intraerythrocytic-stage parasites develop into male and female gametocytes, which can be ingested by a mosquito during feeding. In the mosquito sexual reproduction, followed by further parasite development, leads to a new generation of infectious sporozoites.

CHAPTER 1

Identification and evaluation of anti-*Plasmodium falciparum* antibody response kinetics that are informative of recent and cumulative malaria exposure

ABSTRACT

The acquisition and decay of antibody responses to malaria antigens has only been evaluated for a very small percentage of the total *Plasmodium* proteome. An improved understanding of the dynamics of antibody response kinetics could accelerate the development of accurate tools for malaria sero-surveillance. A protein microarray containing ~23% of the *P. falciparum* proteome was used to probe plasma, taken both immediately before and after the 6-month malaria season, from Malian individuals between the ages of 2-10 years and 18-25 years. Previous analyses used this data set to identify responses to *P. falciparum* antigens that were associated with protection. Here, we performed a secondary analysis of these data to identify responses associated with exposure, as defined by increases with age, a surrogate for cumulative exposure, seasonality, or predictions of days since last infection. Kinetics of antibody acquisition over time and decay following an infection were then investigated for the sets of antigens selected in these analyses. If validated in other transmission settings, this approach could be a useful strategy for identifying novel biomarkers of malaria exposure and improving understanding of the humoral response to *P. falciparum*.

INTRODUCTION

Malaria, caused by infections with *Plasmodium* species, remains one of the leading causes of morbidity and mortality in tropical and subtropical regions. *P. falciparum* is responsible for the majority of deaths attributed to malaria. In regions with the highest intensity of *P. falciparum* exposure, infants have the highest risk of severe and fatal malaria. Non-sterilizing immunity, which protects against clinical disease but not against parasitemia, develops over years with repeated exposure to *P. falciparum* (122). In endemic areas, individuals who survive past a certain age are unlikely to develop severe disease or die from malaria, even though they are continuously re-infected. Experiments that passively transferred antibodies from immune adults into individuals infected with *P. falciparum* have demonstrated that antibodies have a key role in mediating immunity to malaria (123–125). However, precise knowledge of the nature of antibody responses (i.e., specific target antigens, rates of antibody acquisition, and the longevity of responses) to *P. falciparum* is limited (126).

The close association between antibody levels and recent exposure may enable the evaluation of responses to certain *P. falciparum* antigens to identify individuals with the highest level of *P. falciparum* exposure. Responses to specific *P. falciparum* antigens may act as good biomarkers of malaria exposure, and could be utilized by malaria control programs to monitor changes in exposure over time, to evaluate the impact of control interventions, and/or to identify populations most at risk of infection. However, in order to develop serologic assays that are informative about malaria exposure, more knowledge about the acquisition of responses against specific *P. falciparum* antigens—relative to exposure, age, and immunity—is needed (118). Kinetics of antibody responses to specific malaria antigens have only been evaluated for a small subset of antigens, but have been shown to differ (97–99). Little is known about the early acquisition of anti-malarial antibodies in young children or about how these responses compare in older, more immune individuals (127).

The *P. falciparum* genome is estimated to encode over 5200 putative proteins (9), which are differentially expressed during different stages of the parasite's complex life cycle. Despite decades of research, only a handful of immunodominant *P. falciparum* antigens (representing less than 0.5% of the entire genome) were evaluated as potential diagnostic targets during the pre-genomic era (128). The *P. falciparum* genome has been sequenced and the *P. falciparum* proteome has been finalized (9, 129–134), providing the opportunity to utilize high-throughput approaches to identify novel *P. falciparum* antigens for diagnostic applications and to gain a better understanding of immunologic host-parasite interactions. Several studies have used protein microarrays for the high-throughput analysis of vast amounts of proteomic data and large numbers of patient samples to identify humoral correlates of protection from malaria (99, 128, 135–137). Here, we re-analyzed previously published array data from one of these microarray studies (135) to investigate what information specific antibody responses can provide on recent or cumulative *P. falciparum* exposure. We investigated the kinetic profiles of responses against selected *P. falciparum* antigens, and examined potential biological factors underpinning the antibody kinetics.

MATERIALS & METHODS

Ethical Approval. The Ethics Committee of the Faculty of Medicine, Pharmacy, and Odonto-Stomatology and the Institutional Review Board at the National Institute of Allergy and Infectious Diseases, National Institutes of Health approved the cohort study. The Committee for the Protection of Human Participants at the University of California, Berkeley granted an exemption for this secondary analysis of previously published microarray data (135).

Study Site, Participants, and Clinical Endpoints. Samples for this investigation were obtained from participants enrolled in a cohort study carried out in Kambila, Mali. The study population was an age-stratified random sample of 15% of all individuals living in the community. The details of this study have been described elsewhere (138). Malaria transmission at this site is highly seasonal, with a sharp demarcation between the 6-month malaria season categorized by intense *P. falciparum*

exposure and the 6-month dry season during which there is little to no exposure (Figure 1.1).

In May 2006, just before the malaria season, individuals between the ages of 2-10 years and 18-25 years were enrolled in the cohort study after random selection from an age-stratified census of the entire village (population 1,500). 151 children and 43 adults who were followed for the entire course of the malaria season, who did not have parasites detected in their baseline (pre-malaria season) plasma sample, and who had protein microarray data available from their pre-malaria season and/or their post-malaria season plasma sample were included in this analysis. Participants were followed for 8 months subsequent to enrollment, and were instructed to report symptoms of malaria at the village health center. Participants who presented with a documented fever ($\geq$37.5˚C) and had any *P. falciparum* parasites detected by blood smear were classified as having clinical malaria for the purposes of this study. All individuals presenting with a blood smear positive for *P. falciparum* and signs or symptoms of malaria were treated according to international standards, regardless of the parasitemia level. In addition, active surveillance for *P. falciparum* infection by blood smear was performed at study enrollment and at scheduled follow-up visits every 2 months during the 6-month malaria season.

For each participant, the number of clinical malaria episodes occurring between enrollment and the collection of the post-malaria season (December 2006) plasma sample was tallied. Time since last infection was calculated as the number of days before the date of post-malaria season plasma collection when *P. falciparum* parasites were most recently detected, if any. Minimum time since last infection was set to 14 days.

Protein Microarray Chip Fabrication, Probing, and Data Normalization. 2,320 whole or partial proteins corresponding to 1,204 unique *P. falciparum* proteins (using 3D7 reference sequence) were selected for inclusion on the protein microarray. ORFs were derived from the *P. falciparum* genomic sequence database (www. plasmodb.org) and selected based on stage-specific transcription or protein expression (129), subcellular localization in the parasite infected red blood cell, secondary protein structure, or documented immunogenicity in humans or animal models. Fabrication of protein microarrays involved (i) PCR amplification of each complete or partial *P. falciparum* ORF, (ii) in vivo recombination cloning, (iii) in vitro transcription/translation, and (iv) microarray chip printing. Processing of plasma samples and detailed methods on the fabrication and probing of arrays to quantify total IgG intensities have been described previously (135). *P. falciparum* peptide antigens ranged from 31 to 1521 (median 590) amino acids in length.

To stabilize the variance in array data, the vsn method (139) was applied to the quantified array intensities. This procedure corrects for nonspecific noise effects by finding maximum likelihood shifting and scaling parameters for each array, such that the variances of each negative control (“No DNA”, where an empty plasmid vector was placed into the transcription/translation reaction) and positive control (human IgG) probes are minimized. This calibration has been shown to be effective on a number of platforms (140–142).

Breadth and Intensity of Antibody Responses. 826 non-reactive *P. falciparum* antigens, for which fewer than 10% of all plasma samples, taken at either the pre- or the post-malaria season timepoint, had responses at least two standard deviations above the mean intensity of the "No DNA" negative control probes, were removed from further analysis. The 1494 reactive *P. falciparum* antigens meeting inclusion criteria for further analysis are listed in Appendix A. For each participant, the breadth of the antibody response was calculated as the proportion of reactive responses against the 1494 *P. falciparum* antigens included in the analysis. Additionally, the mean intensity of the antibody response against each of the 1494 antigens was calculated for each participant. Bonferroni-corrected Mann-Whitney Tests were used to compare mean breadth and intensity of response among participants stratified by age.

Identification and Evaluation of Responses Informative of Exposure. Responses that were most informative of cumulative or recent *P. falciparum* exposure were identified based on their associations with three key metrics in each participant: incidence of malaria in the prior year and time since last infection, as defined above, and difference in antibody intensity in post-malaria season samples as compared to pre-season responses. To identify antibody responses to specific malaria antigens that correlated with cumulative (long term) exposure to *P. falciparum,* linear regression was used to identify associations between antibody response and age (used here as a surrogate for lifetime malaria incidence) for each antigen. In order to select antigens that induced antibody responses that were stable over time and did not fluctuate over the course of the transmission season, responses detected in pre- and post-malaria season plasma samples were included together in the models. Serologic markers of cumulative exposure were restricted to antigens inducing antibody responses that increased with increasing age. To identify antibody responses to specific malaria antigens that correlated with recent (short term) exposure to *P. falciparum,* antigens whose responses decayed predictably after *P. falciparum* infection were identified using linear models to identify correlation between post-malaria season antibody intensity and the number of days since infections were last detected. Serologic markers predicting the number of days since an individual was last infected with *P. falciparum* were restricted to antigens inducing antibody responses that decreased as days since infection increased. A second metric used to identify antigens associated with recent *P. falciparum* exposure involved investigating seasonal variation by comparing antibody responses in pre- and post-malaria season with paired t tests, and restricted to serologic markers whose antibody responses were higher in post-malaria season samples. To account for multiple comparisons, responses were only considered to be significantly associated with an exposure metric when q values (Benjamini–Hochberg False Discovery Rate-corrected p values) were ≤ 0.05 for that antigen. For each of the three exposure metrics, the Top 1% and 5% of antigens, ranked by R^2 for cumulative exposure and time since infection analyses and ranked by q value for seasonal variation analyses, were selected for further evaluation.

The proportion of reactive proteins (and of the Top 1% and 5% of selected antigens) expressed during different stages of the *P. falciparum* life cycle was ascertained by retrieving genome-wide mass spectrometry data (129, 130, 143–152) from PlasmoDB (www.plasmodb.org). *P. falciparum* orthologs of *P. yoelii* genes

expressed in liver stages were identified (153). Proteins were defined as being expressed during a particular stage of the life cycle if at least 10 spectra, from all studies taken together, mapped to that gene for studies performing mass spectrometry analyses of that life cycle expression stage. Relative protein abundance was determined by calculating the percentage of spectral counts that each gene contributed to the total spectra detected in all genes during each stage of the life cycle, and mean relative abundance was determined for the reactive *P. falciparum* antigens as well as for the Top 1% and 5% of selected antigens. Annotated gene ontology components were downloaded from plasmoDB, and various cellular component categories were determined by electronically annotating the 1494 reactive proteins using grep and regular expressions to search for text patterns in the component terms. The proportion of reactive antigens (and of the Top 1% and 5% of antigens selected by each exposure metric) expressed in in the different cellular component categories was determined. For all analyses of plasmoDB metadata, the top antigens were considered to be over- or underrepresented in each of the various categories—as compared to all reactive antigens on the array—if two-tailed q values were ≤0.05. All analyses were performed with R 3.1.0 (154).

RESULTS

Study Populations and Clinical Outcomes. Participants consisted of 151 children, (2-10 years) and 43 adults (18-25 years) from a cohort study conducted in the rural village of Kambila, Mali. Details of the study site have been reported elsewhere (155).

Importantly, the site is characterized by well-defined demarcation between the 6-month dry season (with little to no *P. falciparum* exposure) and the 6-month malaria season (with intense exposure of *P. falciparum*) (Figure 1.1, (138)). The entomological inoculation rate measured in a nearby village was near 0 during the dry season, and there were approximately 50 – 60 infective bites per person per month in October 2000 (155).

Plasma samples were collected from participants before the start of the transmission season. All participants were followed via active and passive surveillance during the transmission season prior to the collection of post-malaria season plasma samples, allowing evaluation of recent exposure. Risk of clinical malaria decreased with age (Figure 1.2, (135)). The percentage of individuals having at least 1 malaria episode was 16% in 18-25 year olds and was 87% in children (Table 1.1). Consistent with the development of immunity in older participants, adults had, on average, a lower number of malaria episodes during the transmission season (median 0 episodes) than children (median 2 episodes). In addition, 63% of adults had an infection with *P. falciparum* detectable by microscopy at some point over the malaria season, while 97% of children had an infection detected. Similarly, a lower proportion of adults had an infection detected within the 30 days prior to the collection of the post-malaria season sample (33%) than children (60%).

***P. falciparum*-Specific Antibody Profiles Showed Decreased Responses with Increased Days Since Infection.** Out of 2,320 *P. falciparum* antigens on the microarray, 1,494 induced antibody responses that met minimal reactivity criteria for inclusion. Visualization of individual participants' antibody profiles across ages showed an increase in antibody reactivity amongst older subjects (Figure 1.3). Across all age groups, overall antibody reactivity increased over the course of the malaria season. In pre-malaria season plasmas, the overall breadth and intensity of anti-*P. falciparum* antibody responses were comparable between different age groups in children (Figure 1.4). However, the breadth and intensity of pre-malaria season responses were significantly lower in children than in adults ($p<0.005$). This differential response between children and adults was no longer apparent after the malaria season. Amongst children, recent *P. falciparum* exposure was associated with a greater breadth and intensity of response (Figure 1.4, $p<0.05$ for all comparisons of post- vs. pre-malaria season intensity in children). This increase in response was not seen in adults ($p=0.1$ for both breadth and intensity). Since antibody responses in children, both before and after the transmission season, were similar across all age groups, data from 2-10 year old participants were combined for subsequent analyses. As antibody responses in adults did not correlate with recent *P. falciparum* exposure, we approached serologic biomarker discovery by exploring relationships between our exposure metrics and antibody responses in children only.

Antibody Responses Most Predictive of *P. falciparum* Exposure. Using plasma samples from children, we sought to find responses against *P. falciparum* antigens that were most informative of prior malaria exposure. To evaluate responses predictive of cumulative *P. falciparum* exposure, we used the age of each participant as a surrogate for lifetime exposure. Linear regression of pre- and post-malaria season responses determined that 24% of the reactive *P. falciparum* antigens on the array induce responses that, in children, increase predictably with age (Figure 1.5, top). Amongst children who had both pre- and post-malaria season plasmas available for study, paired t-tests were used to identify responses associated with a seasonal change in antibody intensity. The vast majority (98%) of the reactive *P. falciparum* antigens induce responses that increase over the course of the malaria season (Figure 1.5, middle). Linear regression of post-malaria season plasmas determined that 7% of reactive antigens induce responses that are predictive of the number of days since a child was last infected with *P. falciparum* (Figure 1.5, bottom). For all three metrics used to evaluate *P. falciparum* exposure, q-values (False Discovery Rate-corrected q-values) of ≤ 0.05 were considered significant. The top *P. falciparum* antigens inducing responses associated with exposure in children (as assessed by R^2 for the cumulative exposure and time since last infection metrics, and by mean difference in antibody intensity for the seasonal change metric) were investigated further.

Responses to the top antigens selected for predicting all three exposure metrics were generally high. The top antigenic markers for cumulative *P. falciparum* exposure induced responses that followed the expected trend of increasing intensity with age (Figure 1.6, top). Seasonal variation in response was less noticeable for the top antigens predicting cumulative exposure than for the reactive antigens overall. Although the majority of reactive *P. falciparum* antigens induced responses that increased over

the course of the malaria season, the top antigenic markers induced a much more pronounced increase in intensity in post-malaria season samples (Figure 1.6, middle). This seasonal effect was the greatest amongst the youngest children (2-4 years), and declined with increasing age. Although no trend between antibody intensity and time since infection was apparent when looking at all reactive antigens, the top antigenic markers for time since *P. falciparum* infection induced responses, in children, that followed the expected trend of decreased intensity over time (Figure 1.6, bottom). Notably, this trend was absent amongst adults.

Top antigens associated with the three exposure metrics of interest are listed in Tables 1.2-1.4. Amongst responses commonly used in the past to evaluate exposure, AMA1 was not selected by any exposure metric, MSP1 was only associated with seasonal changes in antibody intensity, and CSP was only associated with cumulative exposure. There was no overlap in the Top 1% of antigens selected in each of the three metrics for inducing responses that were predictive of exposure. Conversely, amongst the Top 5% of responses, 11% of selected antigens were chosen in analyses with all three exposure metrics (Figure 1.7). 88% of responses found to be significant by all three metrics corresponded to different members of the highly polymorphic PfEMP1 protein family. However, it is interesting to note that the PfEMP1 peptide fragment selected in each corresponded to the conserved region exon 2, which encodes the intracellular cytoplasmic tail of PfEMP1 (156). The erythrocyte-binding portion of the vaccine candidate EBA175 protein was also selected by all three exposure metrics. As metrics evaluating the correlation between responses and days since last infection or seasonal variation in antibody intensity were thought to be alternative approaches to investigating the half-lives of specific responses, we were surprised that only 20% of the Top 5% of *P. falciparum* antigens selected by these two metrics overlapped. Perhaps more odd is the fact that 17% of the Top 5% of *P. falciparum* antigens selected for associations with cumulative exposure and seasonality overlapped, as these two metrics were expected to be in opposition. Conversely, 35% of the Top 5% of *P. falciparum* antigens selected for associations with cumulative exposure and days since infection overlapped.

From the individual profiles of antibody responses induced by the Top 1% of *P. falciparum* antigens that are associated with cumulative exposure, it is evident that antibodies against specific antigens are acquired to different maximum intensities (Figure 1.8). For example, MSP4 (PF3D7_0207000) is highly immunogenic and induces strong antibody responses even in pre-malaria season samples from the youngest children, while NOT1 (PF3D7_1103800) elicits only weak responses in adults. Additionally, responses are acquired at varying rates. Responses against the two VAR protein hits (PF3D7_080600 and PF3D7_0617400) continue to be steadily acquired through early adulthood. Conversely, responses to other *P. falciparum* proteins, such as PF3D7_1315200 and PF3D70801500, reach a maximum intensity plateau in 8-10 year old subjects. Unlike responses to the top cumulative exposure antigenic markers, which lacked major seasonal variation in antibody intensity, individual profiles of antibody responses induced by the Top 1% of *P. falciparum* antigens associated with a seasonal increase in response demonstrate wide fluctuations in intensity in pre- and post-malaria season plasmas (Figure 1.9). The seasonal increase in response seen in the post-malaria season is most pronounced amongst the youngest children (2-4 years). The

Top 1% of antigens predictive of time since last *P. falciparum* infection demonstrate decreased antibody intensity with increased time in children, but not in adults (Figure 1.10).

Analysis of cellular component data indicate that the top antigenic markers of exposure are generally more likely to be located in either the membrane of the parasite or in the membrane of the host cell (Figure 1.11, top). Antigens inducing responses that are seasonally variable are also likely to be exported to the host cell (Figure 1.11, top) and are also more likely to be expressed in intra-erythrocytic stages of the *P. falciparum* life cycle (Figure 1.11, middle). Finally, seasonally variable responses are more likely to be induced by more abundantly expressed antigens (Figure 1.11, bottom).

DISCUSSION

Using a protein microarray representing ~23% of the *P. falciparum* genome allowed for the identification of antibody responses to antigens associated with *P. falciparum* exposure. Important insights into understanding the kinetics of these selected antigens are presented here. Even though immunity to symptomatic malaria was starting to develop in 8-10 year old Malian children, broad antibody profiles to the reactive *P. falciparum* antigens were overall similar to younger, non-immune children. Young adults, however, demonstrated vastly different patterns with regards to response breadth and intensity. A large proportion of responses to reactive *P. falciparum* antigens increased steadily with age. Responses to certain antigens plateaued in childhood, while responses to other antigens continued to increase through early adulthood. An overwhelming majority of reactive array antigens demonstrated a significant increase in antibody intensity in post-malaria season samples taken from children. However, this relationship between antibody intensity and malaria season was not evident in adult serum. A small subset of responses were predictive of days since last *P. falciparum* infection, and antigens selected in this analysis demonstrate differential antibody decay profiles.

Antigens selected in all three analyses (i.e., having a stable increase with age, high seasonal variation, and/or a consistent decrease over time following an infection) were likely to be expressed on the parasite membrane or to be exported to the host cell. Antigens selected for maximal change in antibody intensity between low- and high-transmission season were more likely to be expressed in intra-erythrocytic stages of the *P. falciparum* life cycle and were also more likely to be abundantly expressed. Interestingly, and consistent with which the way they were selected, antigens inducing seasonal responses seem to be both highly immunogenic and have short half-lives.

A recent proteomics study in The Gambia compared saliva from children with symptomatic malaria to *P. falciparum*-negative children with fever to identify potential biomarkers of recent exposure. Three *P. falciparum* antigens were exclusively identified in malaria patients (157). Two of these antigens, PF3D7_0818900 (HSP70) and PF3D7_0917900 (HSP70-2) were included on the protein microarray utilized here. Both of these antigens were selected in our analyses as top seasonally variable antigens, but were not found to be amongst the top predictors of days since last *P. falciparum*

infection. Even though analyses of seasonal response and predictions of days since last infection were both hypothesized to select for biomarkers of recent malaria exposure, there was little overlap between the antigens selected in these two analyses.

The antigens selected by the three metrics of malaria exposure we evaluated represent potential biomarkers that could be useful in assays for sero-surveillance. In the Malian population studied here, responses to *P. falciparum* antigens were vastly different in children than in adults. The population under study resides in an area of seasonal, but intense, malaria exposure, and it appears that the intensity of specific antibody responses in adults are no longer directly tied to the number or timing of recently-occurring *P. falciparum* infections (i.e., those occurring within the last year). In this epidemiological setting, the biomarkers identified only provide useful information about recent *P. falciparum* infections in children. However, evaluating responses to these antigenic biomarkers in children could be used to obtain an estimate of malaria exposure for the population as a whole. It is possible that the acquisition of anti-malarial antibodies could be influenced by differences in the intensities or patterns of malaria exposure, host and parasite genetics, local vector ecology and parasite transmission dynamics, coverage of barriers to mosquitos, and additional other population factors. We hypothesize that similar correlations between recent exposure and responses to these biomarkers would be observed in adults in low-transmission settings. As accurate measurement of exposure is essential for malaria control and elimination, it will be important to assess the utility of responses to these selected antigens in other exposure settings and age ranges.

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Table 1.1. Descriptive statistics of the study sites and participants.

	Children	Adults
Number of participants*	151	43
Median age in years (range)	5 (2-10)	20 (18-25)
Female gender	51%	65%
Parasitemic at time of pre-season sample collection, n (%)	0 (0%)	0 (0%)
Parasitemic at time of post-season sample collection, n (%)	24 (16%)	10 (23%)
Participants with ≥1 malaria episode last season, n (%)	131 (87%)	7 (16%)
Median number of malaria episodes last season, n (range)	2 (0-5)	0 (0-1)
Participants with ≥1 *P. falciparum* infection last season, n (%)	147 (97%)	27 (63%)
Participants with ≥1 *P. falciparum* infection in the last 30 days, n (%)	90 (60%)	14 (33%)
Median days since last *P. falciparum* infection**, (range)	25 (0-103)	26 (0-163)

*133 participants provided both a pre- and a post-transmission season sample; 15 provided only a pre-season sample; 3 provided only a post- season sample.

**Only participants who had at least one *P. falciparum* infection recorded in the previous transmission season were included.

Table 1.2. Top *P. falciparum* antigens predicting cumulative exposure.

Rank	Gene ID	Description
Top 1%	PF3D7_0207000 (exon 1)	merozoite surface protein 4 (MSP4)
Top 1%	PF3D7_0305500 (exon 1 segment 3)	conserved Plasmodium protein
Top 1%	PF3D7_0402200 (exon 1 segment 1)	surface-associated interspersed protein 4.1 (SURFIN 4.1), pseudogene (SURF4.1)
Top 1%	PF3D7_0617400 (exon 1 segment 3)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0801500 (exon 1 segment 1)	conserved Plasmodium protein
Top 1%	PF3D7_0808600 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0909500	subpellicular microtubule protein 1 (SPM1)
Top 1%	PF3D7_0922100 (exon 1 segment 2)	ubiquitin-like protein
Top 1%	PF3D7_1103800 (exon 1 segment 2)	CCR4-NOT transcription complex subunit 1 (NOT1)
Top 1%	PF3D7_1128300 (exon 2 segment 1)	6-phosphofructokinase (PFK11)
Top 1%	PF3D7_1129600 (exon 1 segment 2)	phosphatidylinositol-4-phosphate-5-kinase
Top 1%	PF3D7_1315200 (exon 1 segment 2)	conserved Plasmodium protein
Top 1%	PF3D7_1329100 (exon 3 segment 3)	myosin C (MyoC)
Top 1%	PF3D7_1419400 (exon 1 segment 1)	conserved Plasmodium membrane protein
Top 1%	PF3D7_1419400 (exon 1 segment 2)	conserved Plasmodium membrane protein
Top 5%	PF3D7_0206800	merozoite surface protein 2 (MSP2)
Top 5%	PF3D7_0212100 (exon 1 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_0220000 (exon 2 segment 1)	liver stage antigen 3 (LSA3)
Top 5%	PF3D7_0303200 (exon 2 segment 2)	HAD superfamily protein
Top 5%	PF3D7_0304600	circumsporozoite (CS) protein (CSP)
Top 5%	PF3D7_0305500 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0420700 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0420700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0501800 (exon 1 segment 1)	chromosome assembly factor 1 (CAF1)
Top 5%	PF3D7_0511400	conserved Plasmodium protein
Top 5%	PF3D7_0521700 (exon 1 segment 1)	DEAD/DEAH box ATP-dependent RNA helicase
Top 5%	PF3D7_0617400 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)

Table 1.2. Top *P. falciparum* antigens predicting cumulative exposure (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_0617400 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0617400 (exon 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0702400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0711700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0713900 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0716300 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0721100	conserved Plasmodium protein
Top 5%	PF3D7_0731500 (exon 1 segment 2)	erythrocyte binding antigen-175 (EBA175)
Top 5%	PF3D7_0800200 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0800300 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0800300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0802000 (exon 1 segment 2)	glutamate dehydrogenase (GDH3)
Top 5%	PF3D7_0803100 (exon 1 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_0808600 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0903500 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0907200 (exon 1 segment 1)	GTPase activator
Top 5%	PF3D7_0911900 (exon 2)	falstatin (ICP)
Top 5%	PF3D7_0918300 (exon 2 segment 1)	eukaryotic translation initiation factor 3 subunit 5
Top 5%	PF3D7_0927300 (exon 1 segment 1)	fumarate hydratase
Top 5%	PF3D7_1007700 (exon 1 segment 2)	transcription factor with AP2 domain(s) (ApiAP2)
Top 5%	PF3D7_1011800 (exon 1 segment 2)	QF122 antigen
Top 5%	PF3D7_1025500 (exon 2 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1035200	S-antigen
Top 5%	PF3D7_1035700 (exon 1 segment 1)	duffy binding-like merozoite surface protein (DBLMSP)
Top 5%	PF3D7_1100200 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1100200 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)

Table 1.2. Top *P. falciparum* antigens predicting cumulative exposure (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_1100200 (exon 1 segment 3)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1100200 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1110400 (exon 3 segment 1)	asparagine-rich antigen
Top 5%	PF3D7_1113100	protein tyrosine phosphatase (PRL)
Top 5%	PF3D7_1116700 (exon 1 segment 1)	cathepsin C, homolog,dipeptidyl peptidase 1 (DPAP1)
Top 5%	PF3D7_1121800	petidase, M16 family
Top 5%	PF3D7_1130200	60S ribosomal protein P0 (PfP0)
Top 5%	PF3D7_1141100 (exon 1 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1149000 (exon 1 segment 1)	antigen 332, DBL-like protein (Pf332)
Top 5%	PF3D7_1149000 (exon 1 segment 4)	antigen 332, DBL-like protein (Pf332)
Top 5%	PF3D7_1203700 (exon 3)	nucleosome assembly protein (NAPL)
Top 5%	PF3D7_1213400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_1246200	actin I (ACT1)
Top 5%	PF3D7_1254100 (exon 2 segment 1)	stevor
Top 5%	PF3D7_1300300 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1300300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1313600 (exon 1 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1320000 (exon 1 segment 1)	rhoptry protein 2 (PRP2)
Top 5%	PF3D7_1327800 (exon 1 segment 1)	ribose-phosphate pyrophosphokinase
Top 5%	PF3D7_1328500 (exon 1 segment 1)	alpha/beta-hydrolase
Top 5%	PF3D7_1335100	merozoite surface protein 7 (MSP7)
Top 5%	PF3D7_1435300 (exon 1 segment 4)	NAD(P)H-dependent glutamate synthase

Table 1.3. Top seasonally variable *P. falciparum* antigens.

Rank	Gene ID	Description
Top 1%	PF3D7_0220000 (exon 2 segment 2)	liver stage antigen 3 (LSA3)
Top 1%	PF3D7_0501100.1; PF3D7_0501100.2 (exon 3 segment 1)	heat shock protein 40, type II (HSP40)
Top 1%	PF3D7_0501100.1; PF3D7_0501100.2 (exon 4 segment 1)	heat shock protein 40, type II (HSP40)
Top 1%	PF3D7_0501200 (exon 2)	parasite-infected erythrocyte surface protein (PIESP2)
Top 1%	PF3D7_0532300 (exon 2 segment 1)	Plasmodium exported protein (PHISTb)
Top 1%	PF3D7_0702300 (exon 2)	sporozoite threonine and asparagine-rich protein (STARP)
Top 1%	PF3D7_0801000 (exon 2 segment 1)	Plasmodium exported protein (PHISTc)
Top 1%	PF3D7_0818900	heat shock protein 70 (HSP70)
Top 1%	PF3D7_0935900 (exon 2 segment 1)	ring-exported protein 1 (REX1)
Top 1%	PF3D7_1033200 (exon 1 segment 1)	early transcribed membrane protein 10.2 (ETRAMP10.2)
Top 1%	PF3D7_1129100 (exon 1 segment 1)	parasitophorous vacuolar protein 1 (PV1)
Top 1%	PF3D7_1149200 (exon 2 segment 1)	ring-infected erythrocyte surface antigen
Top 1%	PF3D7_1353100 (exon 2 segment 1)	Plasmodium exported protein
Top 1%	PF3D7_1436300 (exon 1 segment 1)	translocon component PTEX150 (PTEX150)
Top 1%	PF3D7_1438100	secretory complex protein 62 (SEC62)
Top 5%	PF3D7_0102200 (exon 2 segment 2)	ring-infected erythrocyte surface antigen (RESA)
Top 5%	PF3D7_0207000 (exon 1)	merozoite surface protein 4 (MSP4)
Top 5%	PF3D7_0207500 (exon 1 segment 1)	serine repeat antigen 6 (SERA6)
Top 5%	PF3D7_0220000 (exon 2 segment 1)	liver stage antigen 3 (LSA3)
Top 5%	PF3D7_0301700 (exon 2 segment 1)	Plasmodium exported protein
Top 5%	PF3D7_0318300 (exon 3 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0420700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0422500 (exon 1 segment 1)	pre-mRNA-splicing helicase BRR2 (BRR2)
Top 5%	PF3D7_0501800 (exon 1 segment 2)	chromosome assembly factor 1 (CAF1)
Top 5%	PF3D7_0532100	early transcribed membrane protein 5 (ETRAMP5)
Top 5%	PF3D7_0532400 (exon 2 segment 1)	Plasmodium exported protein (PHISTb)

Table 1.3. Top seasonally variable *P. falciparum* antigens (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_0617400 (exon 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0702400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0708400 (exon 2)	heat shock protein 90 (HSP90)
Top 5%	PF3D7_0711700 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0711700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0724700 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0731500 (exon 1 segment 2)	erythrocyte binding antigen-175 (EBA175)
Top 5%	PF3D7_0731600 (exon 1 segment 1)	acyl-CoA synthetase (ACS5)
Top 5%	PF3D7_0800200 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0800300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0801000 (exon 2 segment 2)	Plasmodium exported protein (PHISTc)
Top 5%	PF3D7_0808600 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0818200 (exon 3)	14-3-3 protein (14-3-3I)
Top 5%	PF3D7_0900200 (exon 2 segment 1)	rifin (RIF)
Top 5%	PF3D7_0917900 (exon 2)	heat shock protein 70 (HSP70-2)
Top 5%	PF3D7_0929000 (exon 2)	conserved Plasmodium protein
Top 5%	PF3D7_0930300	merozoite surface protein 1 (MSP1)
Top 5%	PF3D7_0935800 (exon 1)	cytoadherence linked asexual protein 9 (CLAG9)
Top 5%	PF3D7_1001000 (exon 2 segment 1)	Plasmodium exported protein (hyp12) (PfJ13)
Top 5%	PF3D7_1004200 (exon 1 segment 1)	conserved Plasmodium membrane protein
Top 5%	PF3D7_1014100	conserved Plasmodium protein
Top 5%	PF3D7_1015900 (exon 2 segment 1)	enolase (ENO)
Top 5%	PF3D7_1023100 (exon 1 segment 4)	dynein heavy chain
Top 5%	PF3D7_1024800 (exon 2 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1035700 (exon 1 segment 1)	duffy binding-like merozoite surface protein (DBLMSP)
Top 5%	PF3D7_1036400 (exon 2 segment 2)	liver stage antigen 1 (LSA1)
Top 5%	PF3D7_1037600 (exon 2 segment 1)	DNA repair helicase rad25

Table 1.3. Top seasonally variable *P. falciparum* antigens (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_1100200 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1116800 (exon 4 segment 1)	heat shock protein 101 (HSP101)
Top 5%	PF3D7_1122900 (exon 2 segment 2)	dynein heavy chain
Top 5%	PF3D7_1126000 (exon 1 segment 2)	threonine--tRNA ligase (ThrRS)
Top 5%	PF3D7_1209400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_1211900 (exon 1 segment 1)	non-SERCA-type Ca2 -transporting P-ATPase (ATP4)
Top 5%	PF3D7_1228600 (exon 1 segment 1)	merozoite surface protein 9 (MSP9)
Top 5%	PF3D7_1238100 (exon 1)	calcyclin binding protein
Top 5%	PF3D7_1252100 (exon 7 segment 1)	rhoptry neck protein 3 (RON3)
Top 5%	PF3D7_1252100 (exon 8 segment 2)	rhoptry neck protein 3 (RON3)
Top 5%	PF3D7_1300300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1320800 (exon 3)	dihydrolipamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex
Top 5%	PF3D7_1335100	merozoite surface protein 7 (MSP7)
Top 5%	PF3D7_1335300 (exon 1 segment 1)	reticulocyte binding protein 2 homologue b (RH2b)
Top 5%	PF3D7_1335300 (exon 1 segment 2)	reticulocyte binding protein 2 homologue b (RH2b)
Top 5%	PF3D7_1354200	inositol-polyphosphate 5-phosphatase
Top 5%	PF3D7_1410400 (exon 1 segment 1)	rhoptry-associated protein 1 (RAP1)
Top 5%	PF3D7_1417200 (exon 1 segment 5)	NOT family protein
Top 5%	PF3D7_1439100 (exon 2 segment 2)	DEAD/DEAH box helicase
Top 5%	PF3D7_1439800 (exon 1)	vesicle-associated membrane protein
Top 5%	PF3D7_1461900 (exon 1 segment 2)	valine--tRNA ligase
Top 5%	PF3D7_1471100 (exon 3 segment 1)	exported protein 2 (EXP2)

Table 1.4. Top *P. falciparum* antigens predicting days since last infection.

Rank	Gene ID	Description
Top 1%	PF3D7_0420700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0617400 (exon 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0710000 (exon 1 segment 1)	conserved Plasmodium protein
Top 1%	PF3D7_0711700 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0800200 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_0808600 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_1037600 (exon 3 segment 1)	DNA repair helicase rad25
Top 1%	PF3D7_1100200 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_1126200 (exon 1 segment 1)	40S ribosomal protein S18
Top 1%	PF3D7_1200300 (exon 2 segment 1)	rifin (RIF)
Top 1%	PF3D7_1203700 (exon 3)	nucleosome assembly protein (NAPL)
Top 1%	PF3D7_1300300 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_1300300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 1%	PF3D7_1439100 (exon 1 segment 2)	DEAD/DEAH box helicase
Top 1%	PF3D7_1469600 (exon 1 segment 4)	biotin carboxylase subunit of acetyl CoA carboxylase (ACC1)
Top 5%	PF3D7_0110700 (exon 1 segment 1)	chromatin assembly factor 1 protein WD40 domain
Top 5%	PF3D7_0206800	merozoite surface protein 2 (MSP2)
Top 5%	PF3D7_0214100 (exon 1 segment 1)	protein transport protein sec31 (SEC31)
Top 5%	PF3D7_0305100 (exon 1 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_0318200 (exon 1 segment 2)	DNA-directed RNA polymerase II
Top 5%	PF3D7_0501800 (exon 1 segment 2)	chromosome assembly factor 1 (CAF1)
Top 5%	PF3D7_0523000 (exon 1 segment 2)	multidrug resistance protein (MDR1)
Top 5%	PF3D7_0704300	conserved Plasmodium membrane protein
Top 5%	PF3D7_0711700 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0713900 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0713900 (exon 1 segment 4)	conserved Plasmodium protein
Top 5%	PF3D7_0731500 (exon 1 segment 2)	erythrocyte binding antigen-175 (EBA175)

Table 1.4. Top *P. falciparum* antigens predicting days since last infection (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_0800200 (exon 1 segment 2)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0800300 (exon 2 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_0813400 (exon 2 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0827800 (exon 1 segment 3)	SET domain protein (SET3)
Top 5%	PF3D7_0903000	conserved protein
Top 5%	PF3D7_0903500 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_0907200 (exon 1 segment 1)	GTPase activator
Top 5%	PF3D7_0907400 (exon 1 segment 1)	ATP-dependent protease ATPase subunit ClpY (ClpY)
Top 5%	PF3D7_0918300 (exon 2 segment 1)	eukaryotic translation initiation factor 3 subunit 5
Top 5%	PF3D7_0918900 (exon 1 segment 1)	gamma-glutamylcysteine synthetase (gammaGCS)
Top 5%	PF3D7_0922100 (exon 1 segment 2)	ubiquitin-like protein
Top 5%	PF3D7_1007700 (exon 1 segment 1)	transcription factor with AP2 domain(s) (ApiAP2)
Top 5%	PF3D7_1008000	inositol polyphosphate kinase (IPK1)
Top 5%	PF3D7_1011800 (exon 1 segment 2)	QF122 antigen
Top 5%	PF3D7_1013500 (exon 1 segment 1)	phosphoinositide-specific phospholipase C (PI-PLC)
Top 5%	PF3D7_1014100	conserved Plasmodium protein
Top 5%	PF3D7_1016500; PF3D7_1016600 (exon 1 segment 1)	Plasmodium exported protein (PHISTc)
Top 5%	PF3D7_1025500 (exon 2 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1037600 (exon 2 segment 1)	DNA repair helicase rad25
Top 5%	PF3D7_1040800 (exon 2 segment 1)	rifin (RIF)
Top 5%	PF3D7_1100200 (exon 1 segment 1)	erythrocyte membrane protein 1, PfEMP1 (VAR)
Top 5%	PF3D7_1116700 (exon 1 segment 1)	cathepsin C, homolog,dipeptidyl peptidase 1 (DPAP1)
Top 5%	PF3D7_1121800	petidase, M16 family
Top 5%	PF3D7_1122400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_1126000 (exon 1 segment 2)	threonine--tRNA ligase (ThrRS)
Top 5%	PF3D7_1135600 (exon 1 segment 2)	conserved Plasmodium protein

Table 1.4. Top *P. falciparum* antigens predicting days since last infection (continued).

Rank	Gene ID	Description
Top 5%	PF3D7_1146800 (exon 2)	conserved Plasmodium protein
Top 5%	PF3D7_1149000 (exon 1 segment 1)	antigen 332, DBL-like protein (Pf332)
Top 5%	PF3D7_1213400 (exon 1 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_1221000 (exon 1 segment 3)	histone-lysine N-methyltransferase, H3 lysine-4 specific (SET10)
Top 5%	PF3D7_1223600 (exon 2 segment 1)	conserved Plasmodium protein
Top 5%	PF3D7_1223600 (exon 2 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1233300	pentatricopeptide repeat protein
Top 5%	PF3D7_1238800 (exon 1 segment 1)	acyl-CoA synthetase (ACS11)
Top 5%	PF3D7_1246200	actin I (ACT1)
Top 5%	PF3D7_1254100 (exon 2 segment 1)	stevor
Top 5%	PF3D7_1302800 (exon 1)	40S ribosomal protein S7
Top 5%	PF3D7_1320800 (exon 3)	dihydrolipamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex
Top 5%	PF3D7_1321300 (exon 1 segment 3)	conserved Plasmodium membrane protein
Top 5%	PF3D7_1340600 (exon 1 segment 1)	RNA lariat debranching enzyme (DBR1)
Top 5%	PF3D7_1368800 (exon 1 segment 2)	DNA repair endonuclease
Top 5%	PF3D7_1408400 (exon 1 segment 1)	DNA-repair helicase
Top 5%	PF3D7_1408400 (exon 1 segment 2)	DNA-repair helicase
Top 5%	PF3D7_1408700 (exon 2 segment 2)	conserved Plasmodium protein
Top 5%	PF3D7_1419400 (exon 1 segment 1)	conserved Plasmodium membrane protein
Top 5%	PF3D7_1419400 (exon 1 segment 2)	conserved Plasmodium membrane protein
Top 5%	PF3D7_1434500 (exon 2 segment 3)	dynein-related AAA-type ATPase
Top 5%	PF3D7_1461900 (exon 1 segment 1)	valine--tRNA ligase

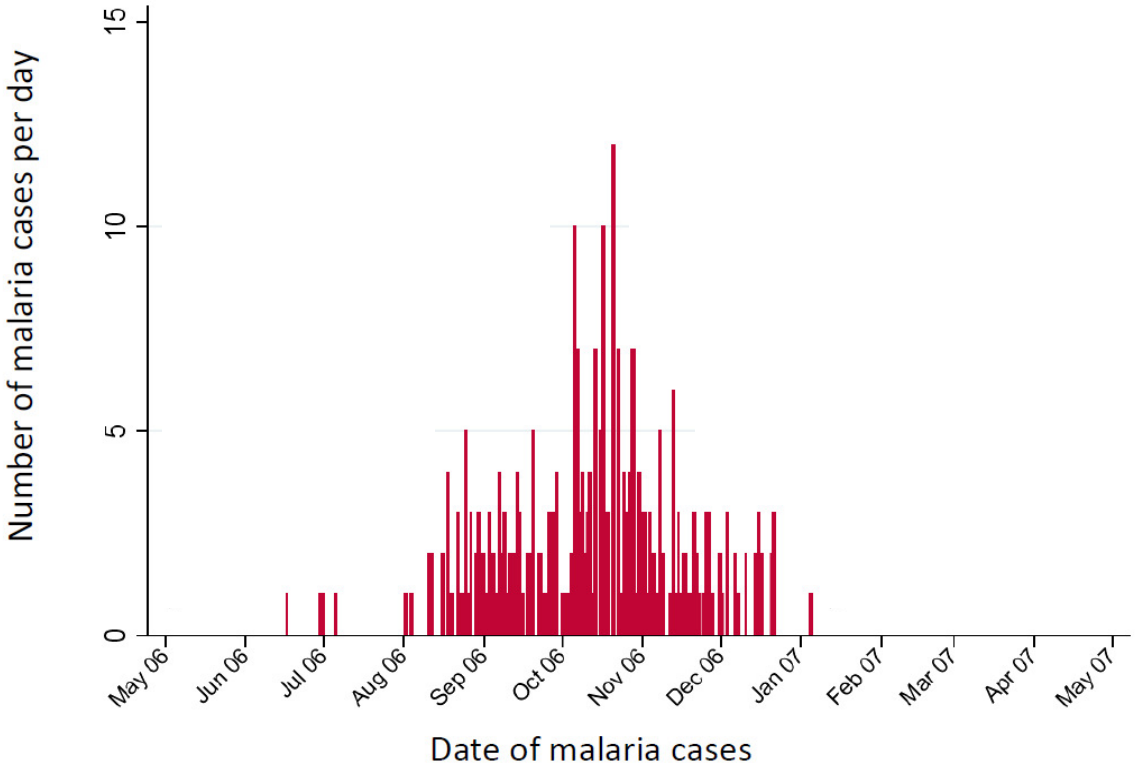

Figure 1.1. Episodes of clinical malaria recorded during study follow-up demonstrate that malaria exposure in Kambila, Mali is highly seasonal. Virtually no transmission of malaria occurs outside of the six-month transmission season, spanning July to January.

Kaplan–Meier estimates of the cumulative probability of malaria during the 6-month malaria season, according to age category.

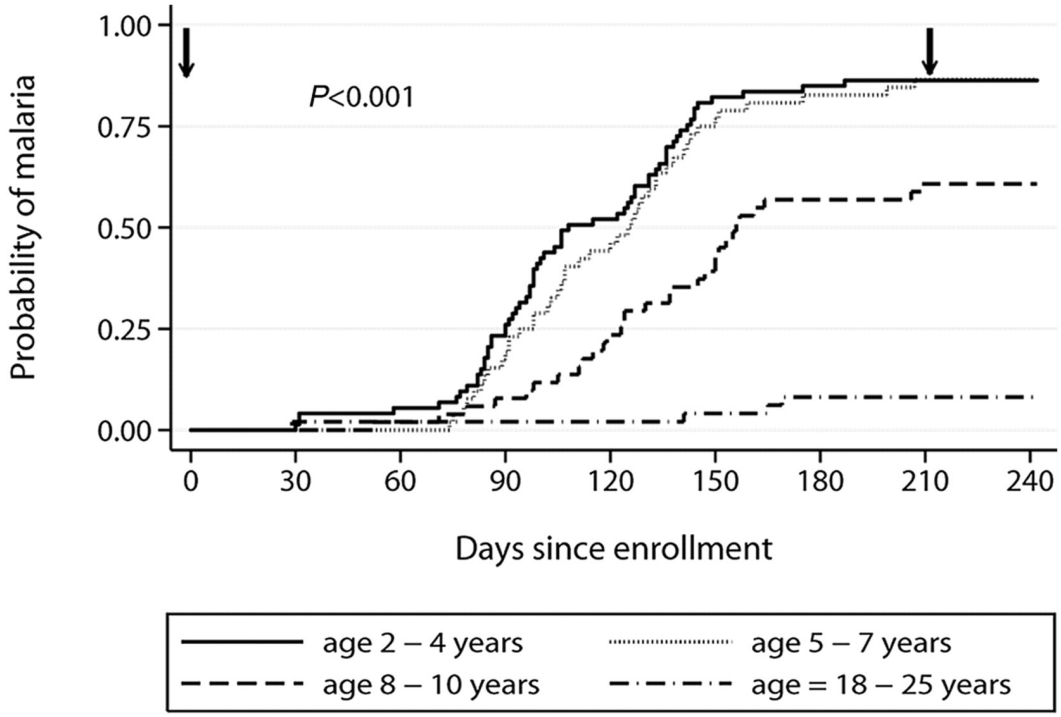

Figure 1.2. Kaplan-Meier estimates of the cumulative probability of malaria during the 6-month malaria season, according to age category. The number of individuals at risk in each age category is shown. Arrows indicate the points at which plasma was collected for protein microarray analysis (before and after the 6-month malaria season). The *P* value was obtained using the log rank test. Figure 1.2 was previously published (135).

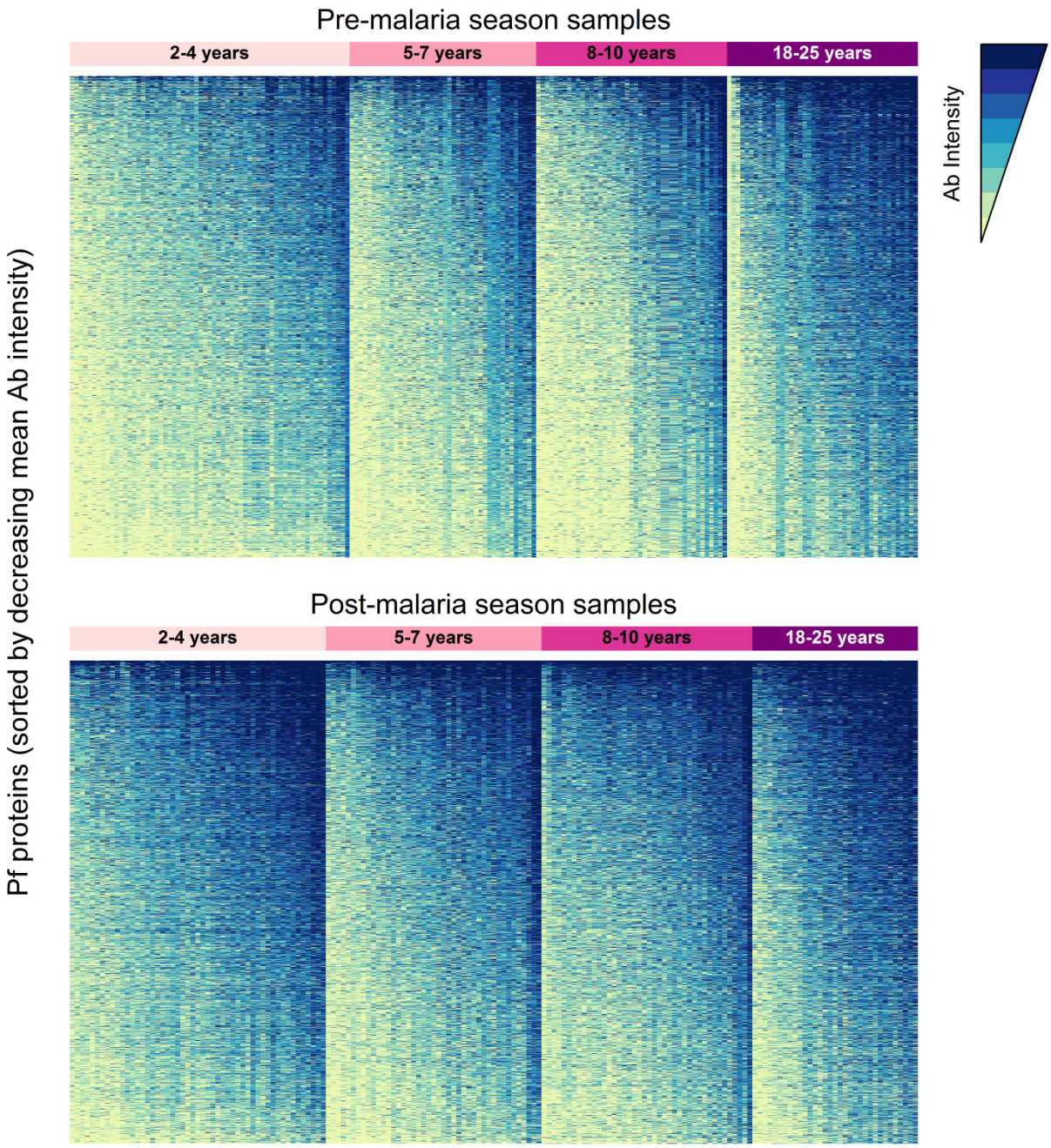

Figure 1.3. Impact of age and malaria exposure on *P. falciparum*-specific antibody profiles. Heat maps of proteins across plasma samples collected before (top) and after (bottom) the 6-month malaria season show that the breadth and intensity of antibody reactivity increases with age and in response to *P. falciparum* exposure. The 1494 reactive proteins are represented in rows in descending order of intensity. Individual plasma samples are in columns and grouped by age (2-4yrs, 5-7yrs, 8-10yrs, and 18-25yrs). Within each age group, samples are sorted by increasing average antibody intensity.

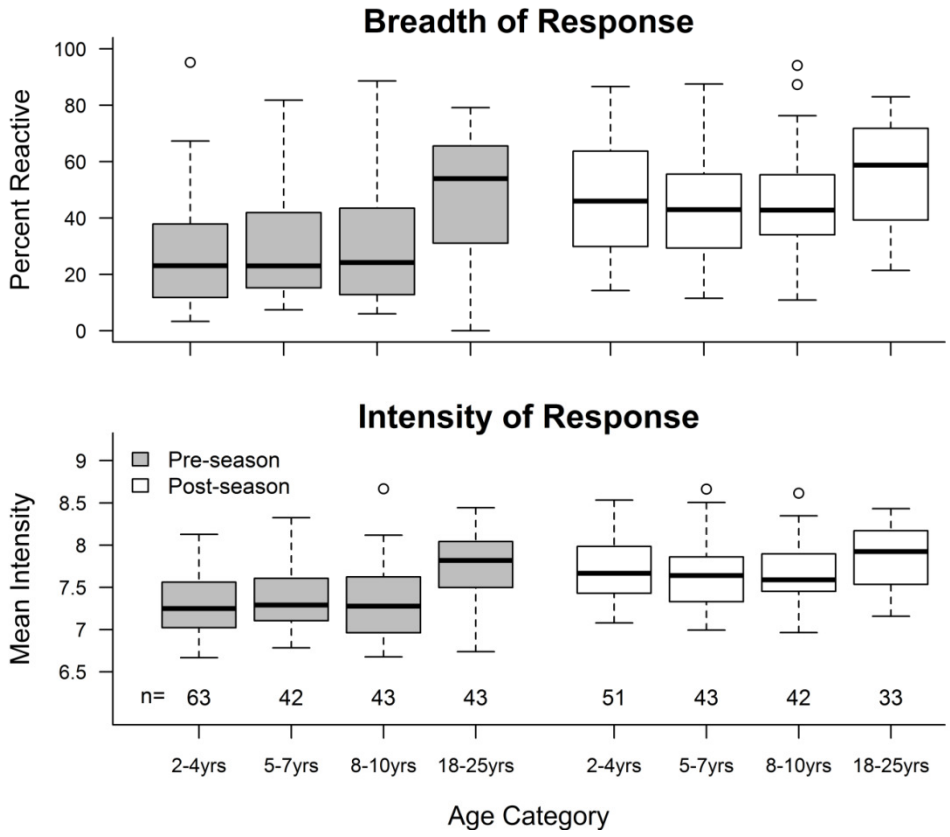

Figure 1.4. Breadth of response for each participant was calculated as the percentage of the 1494 included *P. falciparum* antigens that induced a "reactive" response (where "reactive" is defined as antibody intensity at least 2 standard deviations above empty vector controls). Mean intensity for each participant was calculated from normalized antibody intensities. In children, both the breadth and the intensity of responses were higher in post-malaria transmission season plasmas than in pre-season plasmas in children ($p<0.05$ for all comparisons in children, Mann-Whitney Test with Bonferroni correction). This increase in breadth and intensity in post-malaria season plasmas was not seen in adults. Across age groups, breadth and intensity of responses in both pre- and post-malaria season plasmas did not differ in children. However, amongst pre-malaria season plasmas, adults had significantly higher breadth and intensity of responses than children ($p<0.005$ for all comparisons, Mann-Whitney Test with Bonferroni correction). This difference in breath and intensity between adults and children was not apparent in post-malaria season plasmas.

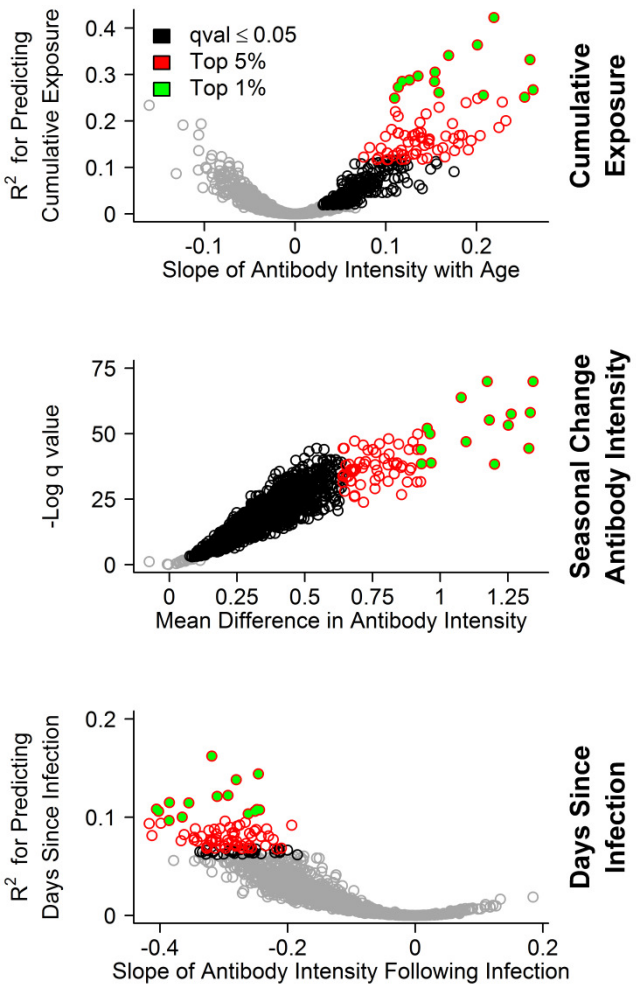

Figure 1.5. Volcano plots, where each dot represents one of the 1494 reactive *P. falciparum* antigens. Key statistics for each of the three exposure metrics were calculated using plasmas from children (2-10 years). **Cumulative exposure (top):** Linear regressions identified responses to antigens that correlated with age in both pre- and post-malaria season samples, a surrogate for cumulative *P. falciparum* exposure and further analysis was restricted to responses that increased with age. Responses to 24% of reactive antigens increase significantly with age. **Seasonal change in antibody intensity (middle):** For each antigen, mean antibody intensity was compared in post- versus pre-malaria season plasma samples with paired t-tests. Responses to 98% of reactive antigens show a significant increase after the malaria season. **Days since infection (bottom):** Linear regressions identified responses in post-malaria season plasmas that significantly correlated with days since an individual was last infected with *P. falciparum.*Responses to 7% of reactive antigens decreased predictably over time since infection. Further characterization of responses was restricted to the top 5% of antigens in each category (points in red), ranked by R^2 for cumulative exposure and days since infection analyses or by intensity increase in post-malaria season plasmas for seasonal change analyses.

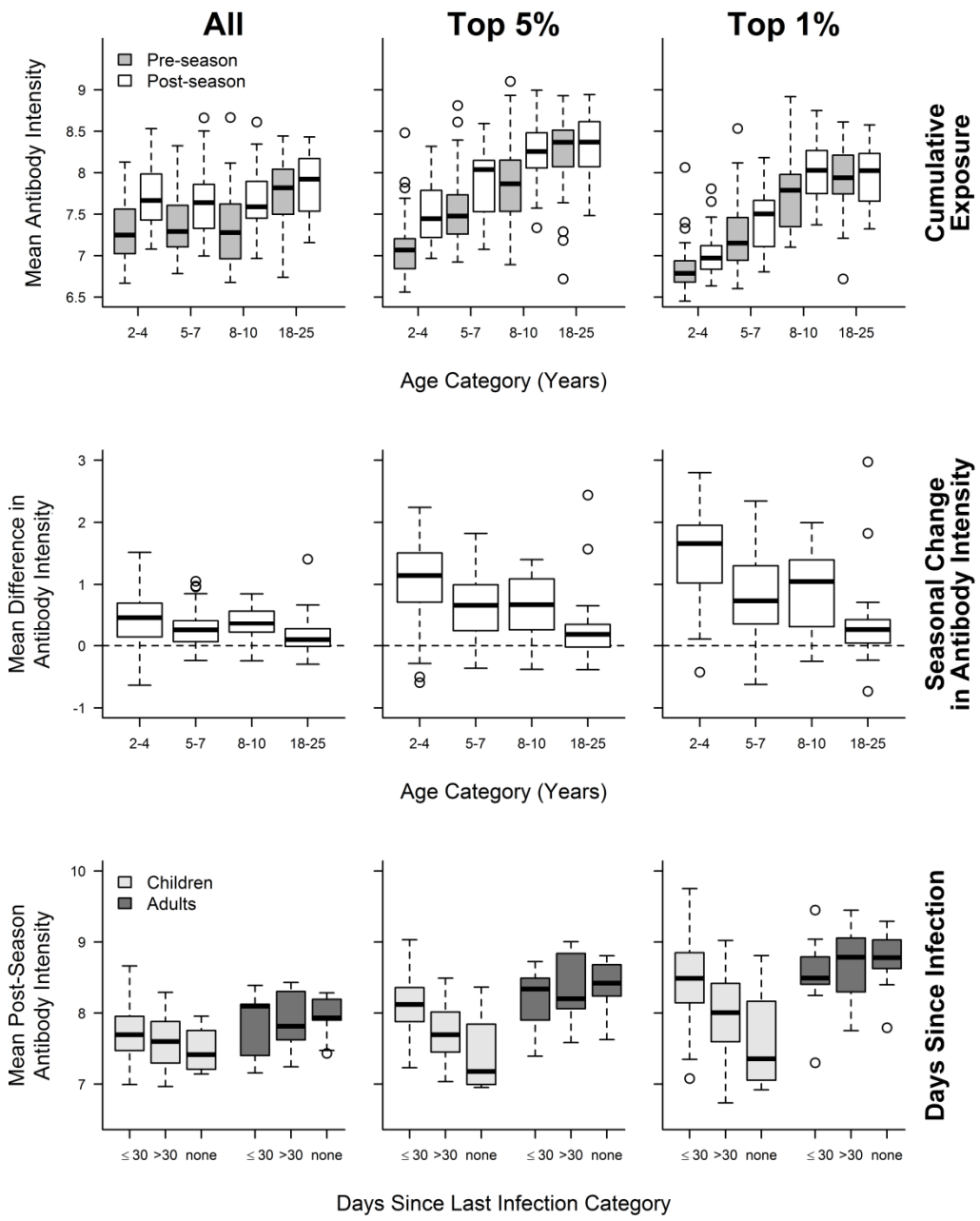

Figure 1.6. Summary boxplots of antibody responses against the 1494 reactive *P. falciparum* antigens (left) and the Top 1% (middle) and 5% (right) of antigens selected in each analysis. Participants were grouped into categories by age (2-4yrs, 5-7yrs, 8-10yrs, 18-25yrs) or time since last *P. falciparum* infection (1: 0 to ≤14d, 2: 15 to ≤30d, 3: 30 to ≤45d, 4: ≥45d, 5: no parasites detected during the previous malaria season). Responses against the top antigens selected for each exposure metric demonstrated more consistent associations with exposure than overall responses.

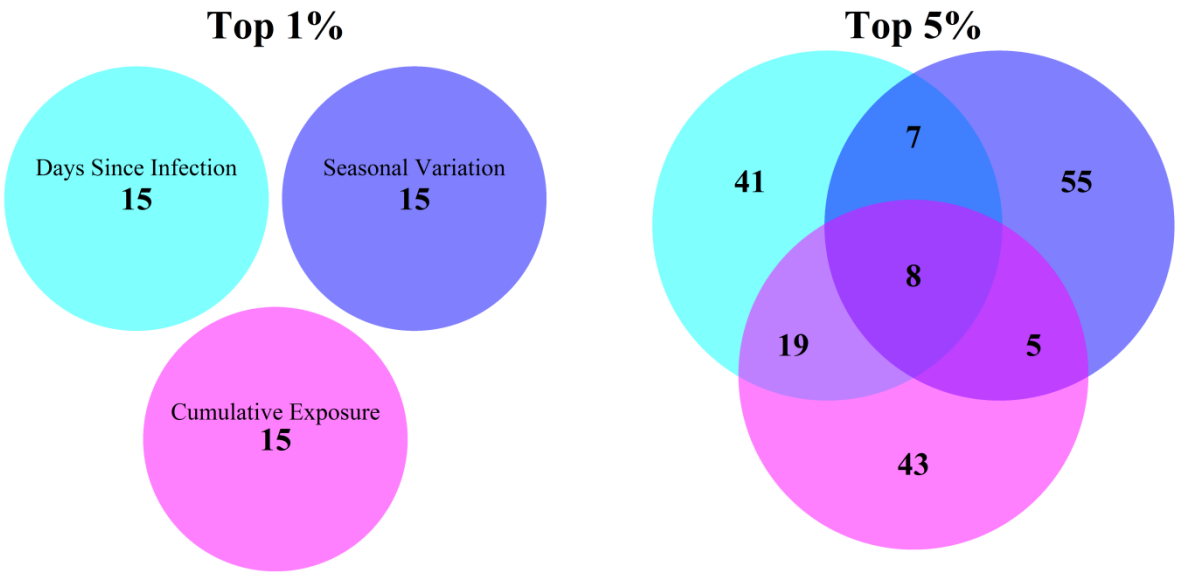

Figure 1.7. Venn diagrams of the Top 1% (left) and 5% (right) of *P. falciparum* antigens selected by each of the three exposure metrics. Although there is no overlap in biomarkers for each metric amongst the Top 1%, many of the Top 5% of biomarkers are associated with multiple metrics of exposure. Amongst the 8 antigens selected for associations with cumulative *P. falciparum* exposure, seasonal variation in antibody intensity, and days since last *P. falciparum* infection, 7 were members of the highly polymorphic *var* (PfEMP1) gene family. All 7 of these PfEMP1peptide fragments mapped to the intracellular domain of the protein, which is highly conserved between PfEMP1proteins.

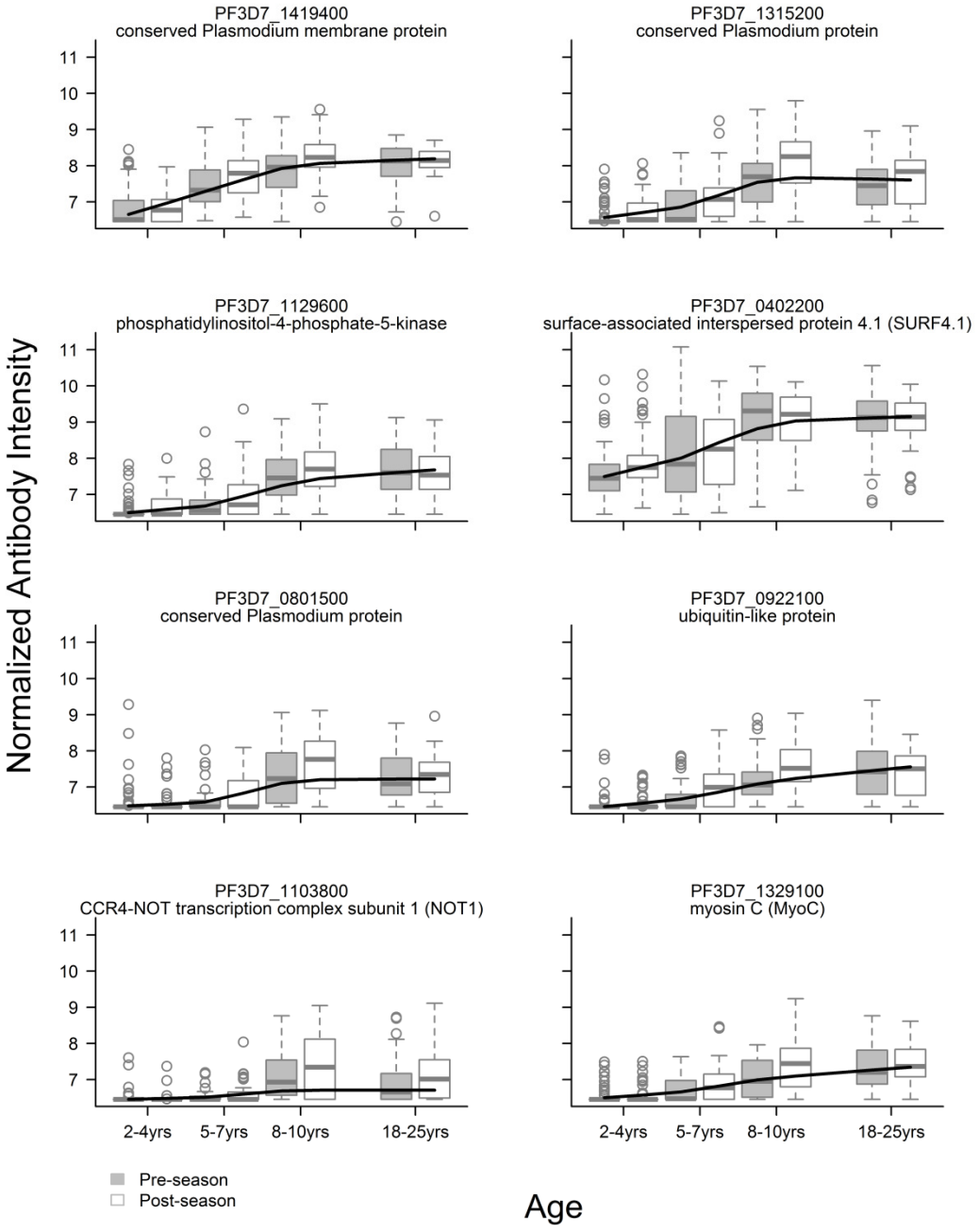

Figure 1.8. Boxplots of antibody intensity (by age category) in pre- and post-malaria season samples for the Top 1% of antigens selected for increased response with age (cumulative exposure). LOWESS smoothing curves representing the increase in intensity over time when responses at both time points were combined indicate that antibody responses to different *P. falciparum* antigens saturate at different levels and are acquired at differential rates.

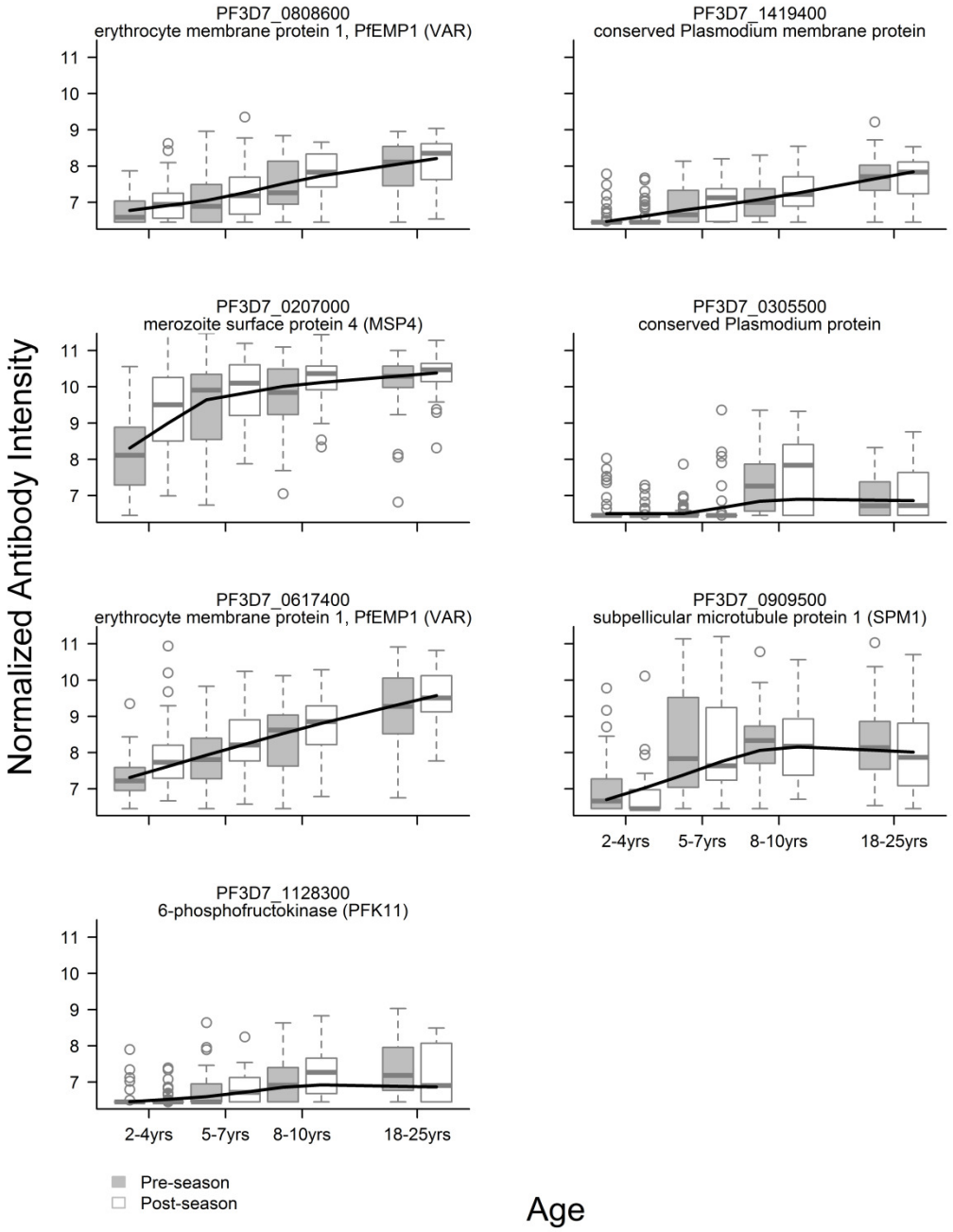

Figure 1.8 (continued). Boxplots of antibody intensity (by age category) in pre- and post-malaria season samples for the Top 1% of antigens selected for increased response with age (cumulative exposure). LOWESS smoothing curves representing the increase in intensity over time when responses at both time points were combined indicate that antibody responses to different *P. falciparum* antigens saturate at different levels and are acquired at differential rates.

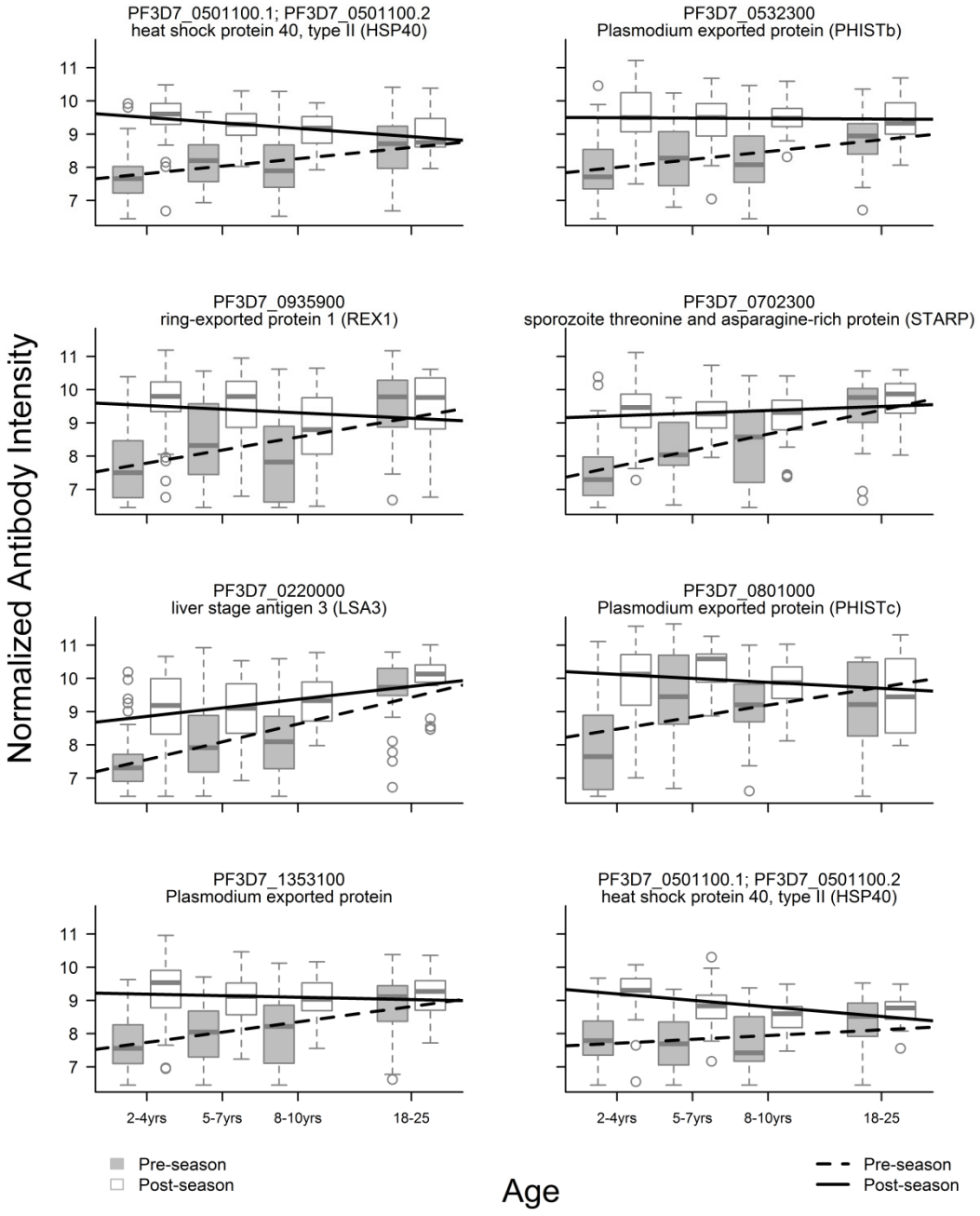

Figure 1.9. Boxplots of antibody intensity (by age category) in pre- and post-malaria season samples for the Top 1% of antigens selected for showing an increase in intensity in post-malaria season samples (Recent Exposure #1). Lines representing the fit of linear models of the antibody response against the *P. falciparum* antigen in pre- or post-malaria season samples indicate that, over the course of the malaria season, the youngest children have the largest acquisition of antibodies against the Top 1% of antigens selected by this exposure metric.

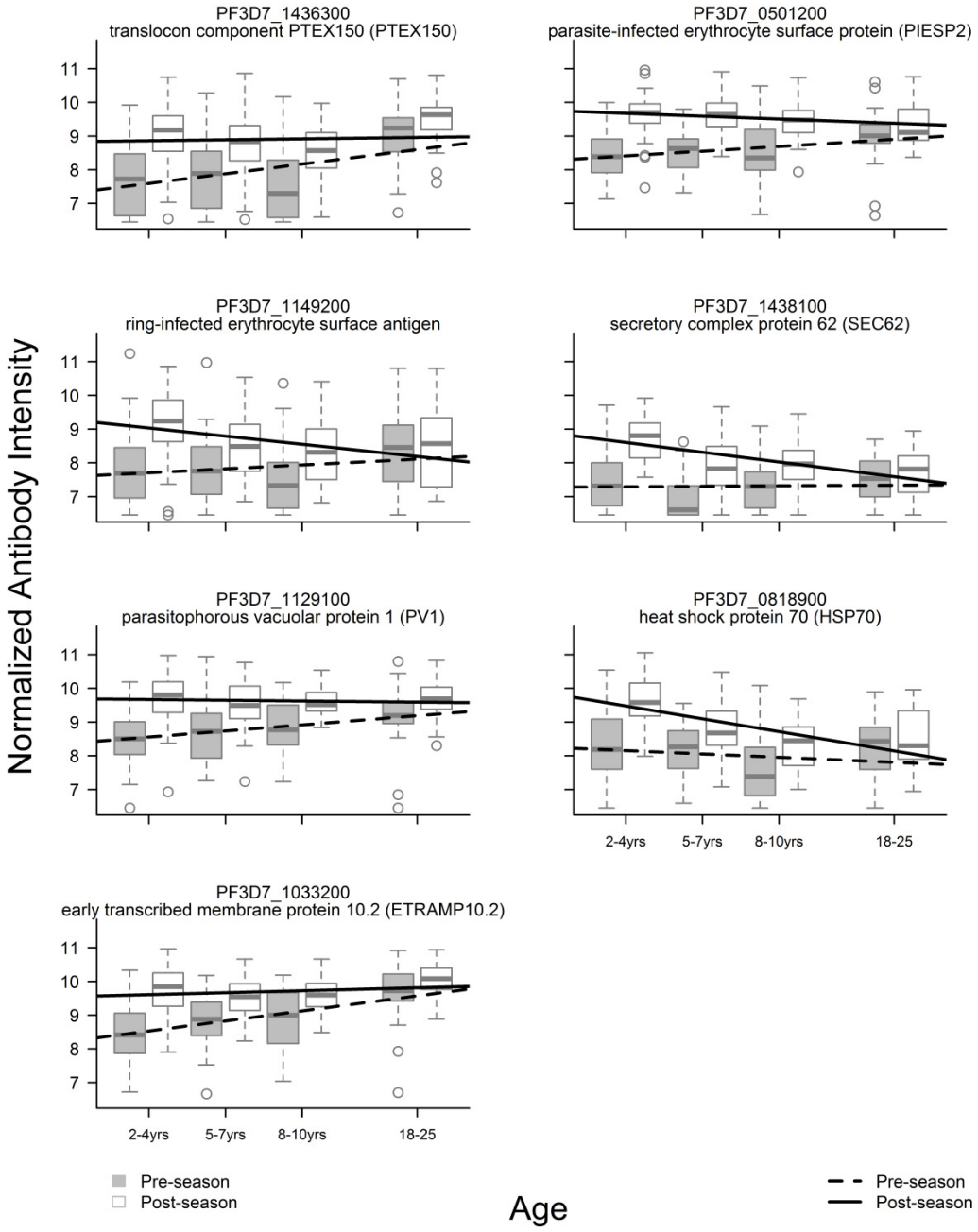

Figure 1.9 (continued). Boxplots of antibody intensity (by age category) in pre- and post-malaria season samples for the Top 1% of antigens selected for showing an increase in intensity in post-malaria season samples (Recent Exposure #1). Lines representing the fit of linear models of the antibody response against the *P. falciparum* antigen in pre- or post-malaria season samples indicate that, over the course of the malaria season, the youngest children have the largest acquisition of antibodies against the Top 1% of antigens selected by this exposure metric.

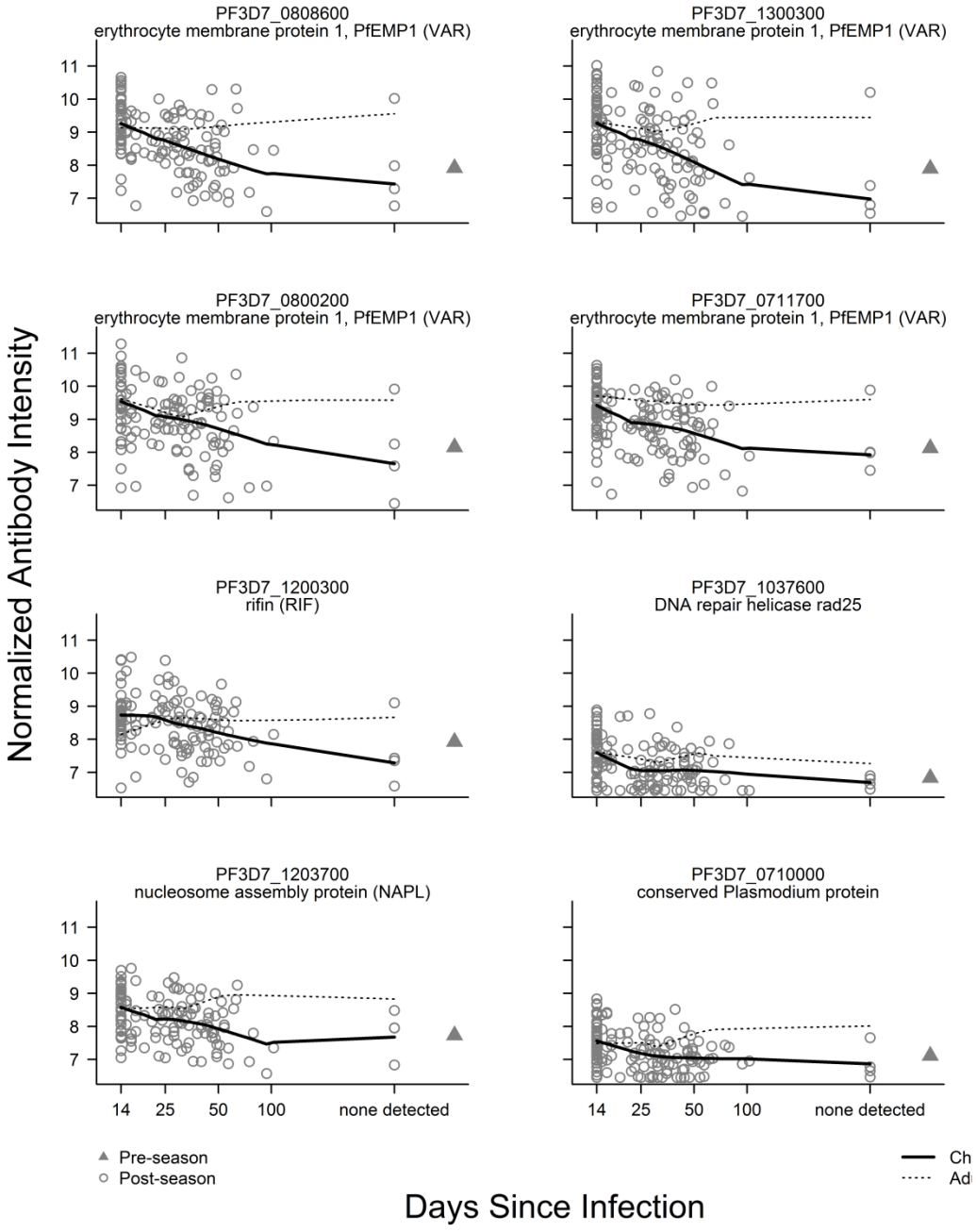

Figure 1.10. For the Top 1% of antigens selected for showing a decrease in response over time since parasites were last detected in children (Recent Exposure #2a), each child's post-malaria season antibody intensity was plotted against the number of days that had elapsed since parasites were last detected. The decrease in antibody response over time that was seen in children (LOWESS smoothing curve, solid line) was not apparent in adults (LOWESS, dashed line).

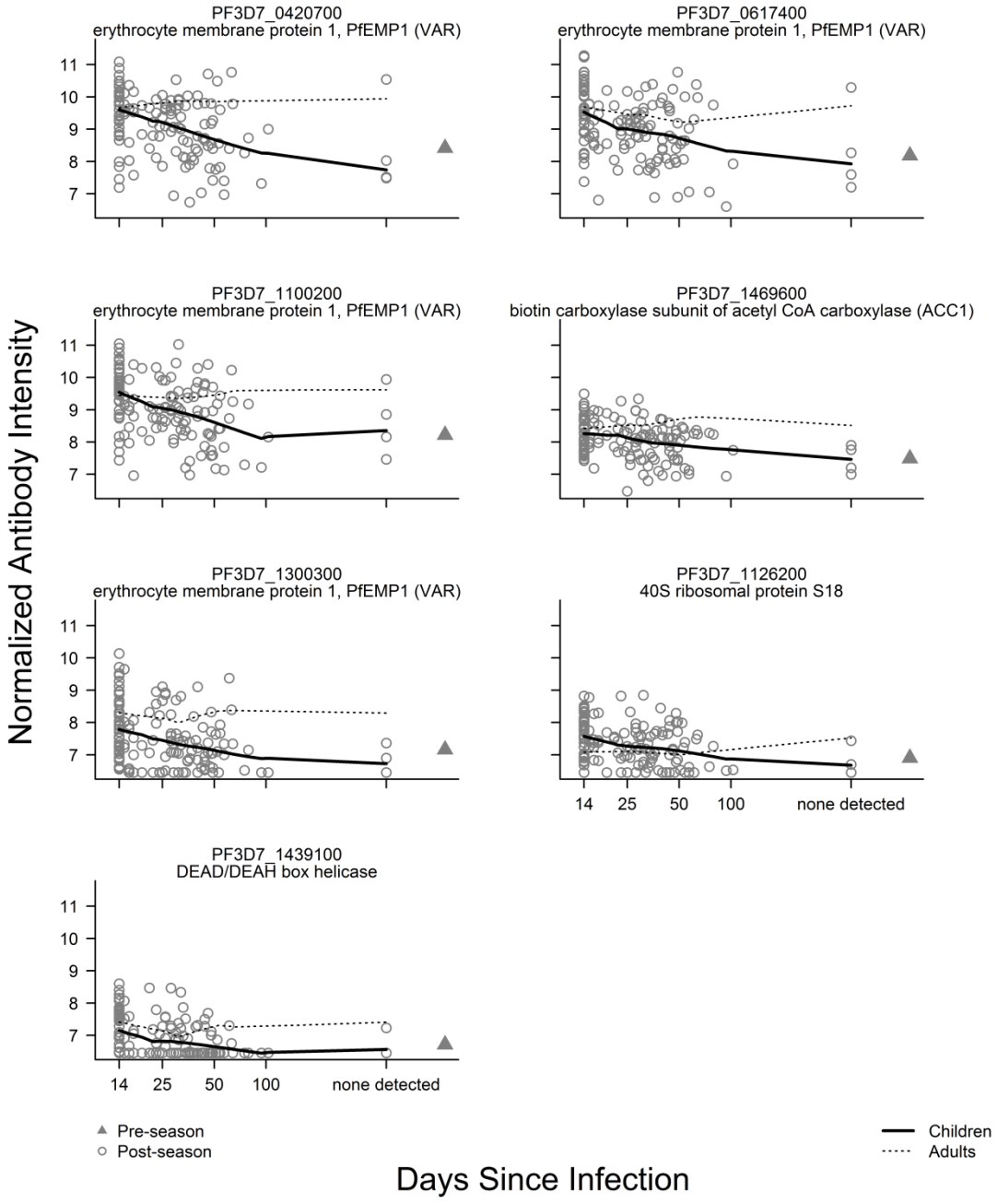

Figure 1.10 (continued). For the Top 1% of antigens selected for showing a decrease in response over time since parasites were last detected in children (Recent Exposure #2a), each child's post-malaria season antibody intensity was plotted against the number of days that had elapsed since parasites were last detected. The decrease in antibody response over time that was seen in children (LOWESS smoothing curve, solid line) was not apparent in adults (LOWESS, dashed line).

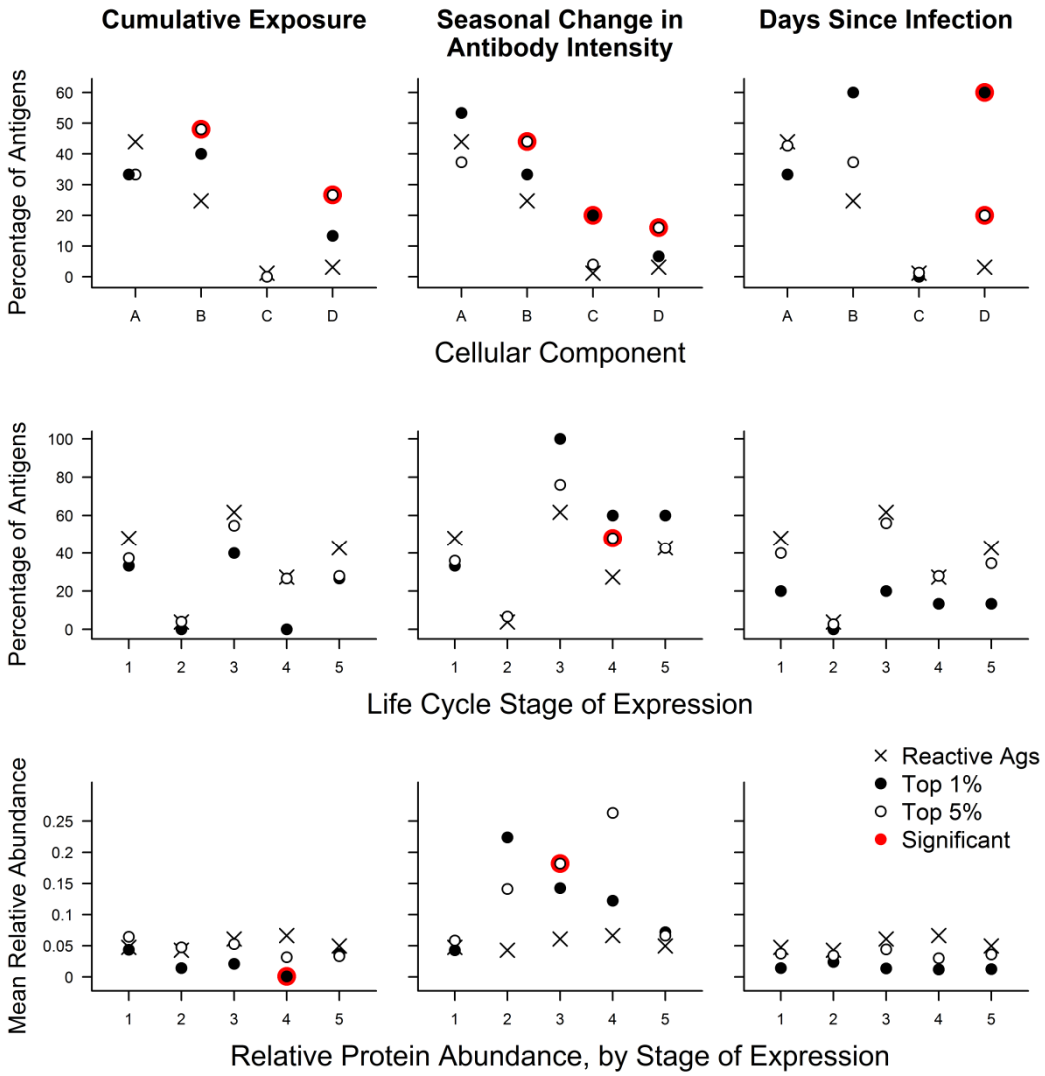

Figure 1.11. *P. falciparum* protein metadata downloaded from plasmoDB for all 1494 reactive array antigens was used to create categories of cellular components, life cycle expression stages, and abundance within each stage of the life cycle. For each metadata analysis, the top antigens selected by each of the three exposure metrics (in columns) were compared to all reactive antigens. The percentages or means of the Top 1% and 5% and all reactive antigens annotated with each category was calculated, and categories represented by a significantly higher percentage of selected antigens were determined. ***Cellular Component (top).*** A: intracellular, B: parasite membrane, C: host cell, D: host cell membrane. ***Life Cycle Stage of Expression (middle).*** 1: sporozoite, 2: liver, 3: intra-erythrocytic, 4: merozoite, 5: gametocyte. ***Relative Protein Abundance, by Stage of Expression (bottom).*** 1: intra-erythrocytic, 2: merozoite, 3: sporozoite, 4: gametocyte, 5: liver.

CHAPTER 2

Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities

ABSTRACT

Tools to reliably measure *Plasmodium falciparum* exposure in individuals and communities are needed to guide and evaluate malaria control interventions. Serologic assays can potentially produce precise exposure estimates at low cost; however current approaches, based on responses to a few characterized antigens, are not designed to estimate exposure in individuals. *P. falciparum*-specific antibody responses differ by antigen, suggesting that selection of antigens with defined kinetic profiles will improve estimates of *P. falciparum* exposure. To identify novel serologic biomarkers of malaria exposure, we evaluated responses to 856 *P. falciparum* antigens via protein microarray in 186 Ugandan children, for whom detailed *P. falciparum* exposure data were available. Using data-adaptive statistical methods, we identified combinations of antibody responses that maximized information on an individual's recent exposure. Responses to three novel *P. falciparum* antigens accurately classified whether an individual had been infected within the last 30, 90, or 365 days (cross validated AUC: 0.86-0.93), while responses to six antigens accurately estimated an individual's malaria incidence in the prior year. Cross validated incidence predictions for individuals in different communities provided accurate stratification of exposure between populations and suggest that precise estimates of community exposure can be obtained from sampling a small subset of that community. In addition, serologic incidence predictions from cross-sectional samples characterized heterogeneity within a community similarly to one year of continuous passive surveillance. Development of simple, ELISA-based assays derived from the successful selection strategy outlined here offers the potential to generate rich epidemiologic surveillance data that will be widely accessible to malaria control programs.

SIGNIFICANCE STATEMENT

We report a new strategy for identifying antigens inducing antibody responses predictive of exposure to *P. falciparum* infection. Our results, generated by screening responses to 856 antigens in children with known *P. falciparum* exposure histories, indicate that responses to a few appropriately selected antigens provide accurate estimates of recent exposure in individuals. This method is distinct from previous

serologic assessments of *P. falciparum* exposure based on community-level responses to a limited number of antigens. Using individual-level serologic estimates of malaria exposure, we accurately characterized fine-scale heterogeneity in exposure within communities and provided precise estimates of exposure in communities from cross-sectional samples. This approach adds an important new tool for infectious disease surveillance that can be translated into high-throughput field-based assays.

INTRODUCTION

Many countries have extensive programs to reduce the burden of *P. falciparum*, the parasite responsible for most malaria morbidity and mortality (158). Effectively using limited resources for malaria control or elimination and evaluating interventions requires accurate measurements of the risk of being infected with *P. falciparum* (25–27, 159–169). To reflect the rate at which individuals are infected with *P. falciparum* in a useful way, metrics used to estimate exposure in a community need to account for dynamic changes over space and time, especially in response to control interventions (31, 170, 171).

A variety of metrics can be used to estimate *P. falciparum* exposure, but tools that are more precise and low-cost are needed for population surveillance. Existing metrics have varying intrinsic levels of precision and accuracy and are subject to a variety of extrinsic factors such as cost, time, and availability of trained personnel (172). For example, entomological measurements provide information on mosquito to human transmission for a community but are expensive, require specially trained staff, and lack standardized procedures, all of which reduce precision and/or make interpretation difficult (172–175). Parasite prevalence can be measured by detecting parasites in the blood of individuals from a cross-sectional sample of a community and is therefore relatively simple and inexpensive to perform, but results may be imprecise especially in areas of low exposure (172, 176) and may be biased by a number of factors including immunity and access to antimalarial treatment (25, 162, 172, 176–178). The burden of symptomatic disease in a community can be estimated from routine health systems data, however such data are frequently unreliable (25, 179–181) and generally underestimate the prevalence of *P. falciparum* infection in areas of intense transmission. Precise and quantitative information about exposure at an individual level can be reliably obtained from cohort studies by measuring the incidence of asymptomatic and/or symptomatic *P. falciparum* infection, i.e. by measuring the molecular force of infection (182–188). Unfortunately, the expense of cohort studies limits their use to research settings. The end result is that most malaria endemic regions lack reliable, timely data on *P. falciparum* exposure, limiting the capabilities of malaria control programs to guide and evaluate interventions.

Serologic assays offer the potential to provide incidence estimates for symptomatic and asymptomatic *P. falciparum* infection, which are currently obtained from cohort studies, at the cost of cross-sectional studies (114, 118, 189). While *P. falciparum* infections are transient, a record of infection remains detectable in an individual's antibody profile. Thus, appropriately chosen antibody measurements, integrated with age, can provide information about an individual's exposure history.

Antibodies can be measured via simple ELISAs and obtained from dried blood spots, which are easy to collect, transport, and store (110, 111, 190). Serologic responses to *P. falciparum* antigens have been explored as potential epidemiological tools (191–194), and estimated rates of seroconversion to well-characterized *P. falciparum* antigens accurately reflect stable rates of exposure in a community while distinct changes in these rates are obtained from successful interventions (110–112, 175, 195–201). However current serologic assays are not designed to detect short term or gradual changes in *P. falciparum* exposure, or to measure exposure to infection at an individual level. The ability to calibrate antibody responses to estimates of exposure in individuals could allow for more flexible sampling of a population (e.g. not requiring age stratification), improve accuracy of exposure estimates from small sample sizes, and better characterize heterogeneity in exposure within a community.

Different *P. falciparum* antigens elicit antibody responses with different magnitudes and kinetics, providing a large and diverse set of potential biomarkers for exposure (118, 127, 202–205). We hypothesized that new and more highly informative serologic biomarkers better able to characterize an individual's recent exposure history could be identified by analyzing antibody responses to a large number of candidate *P. falciparum* antigens in participants with well-characterized exposure histories. To test this hypothesis, we probed plasma from participants in two cohort studies in Uganda against a protein microarray containing 856 *P. falciparum* antigens. The primary aim of this analysis was to identify responses to select antigens that were most informative of recent exposure using robust, data-adaptive statistical methods. Each participant's responses to these selected antigens were used as predictors for two primary outcomes of their recent exposure to *P. falciparum:* (i) days since last *P. falciparum* infection and (ii) the incidence of symptomatic malaria in the last year. These individual-level estimates were then aggregated across a population to assess community-level malaria exposure. The selection strategy presented here identified accurate biomarkers of exposure for children living in areas of moderate to high *P. falciparum* exposure, and illustrates the utility of this flexible and broadly applicable approach.

MATERIALS AND METHODS

Ethical Approval. Written informed consent was obtained from the parent or guardian of all study participants. Ethical approval was obtained from the Uganda National Council of Science and Technology and the institutional review boards of the University of California, Berkeley, the University of California, San Francisco, Makerere University, and the Centers for Disease Control and Prevention. The Tororo Child Cohort (TCC) is registered at ClinicalTrials.gov (NCT00527800).

Study Sites, Participants, and Clinical Endpoints. Samples for this investigation were obtained from Ugandan children enrolled in either the TCC study or from the Kanungu site of the Program for Resistance, Immunology, and Surveillance of Malaria (PRISM) cohort study. For all children included in this analysis, follow-up was complete for the year prior to sample collection. The details of these two longitudinal studies have been described elsewhere (206, 207). Briefly, the TCC study was

conducted from 2007-2012 in Tororo, a rural district in southeastern Uganda with intense perennial exposure (208, 209), where children were enrolled in infancy and followed until 5 years of age. For this study, we included all children born to HIV-negative mothers who remained in the study until 4 years of age and for whom a plasma sample within 4 months of the participant's fourth birthday was available (all collected from November 2010 to November 2011). The PRISM study took place from 2011-2013 in the Kihihi sub-county of Kanungu, a rural district in southwestern Uganda with moderate seasonal exposure (207). For this study, we included all children in the cohort who were 3 to 7 years of age after 1 year of follow-up and had plasma available at this time point (July 2012 to September 2012). For Kanungu participants, GPS coordinates of households were recorded and female Anophelene mosquito counts for each house were determined by monthly CDC light trap counts (208).

Children at both Ugandan study sites were followed for all medical problems with continuous passive surveillance. Children who presented with a documented fever ($\geq$38.0˚C) or history of fever in the previous 24 hours had blood obtained by finger prick for a thick smear. If the thick smear was positive for asexual *P. falciparum* parasites the patient was diagnosed with malaria and given artemisinin-based combination therapy. In addition, active surveillance for parasitemia via thick smear was performed monthly in Tororo and once every three months in Kanungu; children were not treated for parasitemia if asymptomatic. For each participant, malaria incidence in the previous year was calculated as the number of symptomatic malaria episodes occurring in the 365 days prior to sample collection. 14 days of follow-up was removed from total time at risk following every treatment with anti-malarial medication. Days since last infection was calculated as the number of days before the date of plasma collection when *P. falciparum* parasites were most recently detected, if any, in the prior year. When an individual's last detected infection was asymptomatic and a negative smear was obtained in the visit immediately prior, a date falling directly between these two visit dates was used to account for differences in the active surveillance sampling frames between the two sites. For participants who were infected within the two weeks prior to plasma collection, recorded days since last infection was set to 14 to account for the boosting of antibody responses immediately following an infection.

Protein Microarray Chip Fabrication, Probing, and Data Normalization. 856 antigens, corresponding to 520 unique *P. falciparum* proteins, were selected for inclusion based on associations with exposure and immunogenicity in the analyses contained in Chapter 1 and published and unpublished microarray studies conducted at UC Irvine (128, 135, 210). All 178 antigens identified in Chapter 1 were included as probes on this array. In addition, the array contained 40 antigen probes that were dilutions of 12 unique purified *P. falciparum* proteins (211, 212), but none of these probes were selected as informative biomarkers of exposure in any analysis. ORFs from *P. falciparum* 3D7 reference sequences were derived from the *Plasmodium* genomic sequence database (www. plasmodb.org) for the remaining 816 recombinant protein probes on the array. Fabrication of protein microarrays involved (i) PCR amplification of each complete or partial *P. falciparum* ORF, (ii) in vivo recombination cloning in *E. coli*, (iii) in vitro transcription / translation, and (iv) microarray chip printing. Peptide antigens ranged from 50 to 1013 amino acids in length, with a median of 572

amino acids. Processing of plasma samples, which included a 1/200 dilution in buffer containing *E. coli* lysate, and production and probing of arrays to quantify total IgG intensities (optical densities) have been described previously (135).

Data analyses were performed with R 3.1.0 (154). After subtracting slide background, mean empty *E. coli* vector ("No DNA", where an empty plasmid vector was placed into the transcription / translation reaction) intensity was subtracted from each spot to adjust for any cross-reaction effects from the *E. coli* vector used to print the arrays. Next, intensities for each spot underwent inverse hyperbolic sine transformation to yield a Gaussian distribution while avoiding the normalization properties of the variance stabilizing normalization. As individual microarray slides or sample pads might be brighter or darker than others during scanning, leading to biased estimates of antibody intensity, data were normalized after transformation using the robust linear model (213, 214). Essentially, a robust statistical model using the "sandwich estimator" was fit to the data to estimate fixed effects for each slide and each pad based on the negative ("No DNA") and positive (human IgG) control probes. Estimates of the slide and pad effects from the RLM were then subtracted from each probe's intensity to remove any variation solely due to differences among the slides or pads. After RLM normalization, the data was further normalized using a generalized additive model (GAM) to minimize nonlinear differences in antibody intensity detected between two batches of slides, parameterized on a third batch of slides containing a subset of samples from the first two batches. Visual inspection of principle components on samples from all three batches indicated that normalized results showed no appreciable batch effect. Only antigen fragment spots for which transformed and normalized intensity values were higher than 2 standard deviations above the mean of transformed and normalized "No DNA" control spot intensities were analyzed further.

Breadth and Intensity of Antibody Responses. 201 non-reactive *P. falciparum* antigens, for which fewer than 10% of Ugandan children had responses at least one standard deviation above the mean intensity of 28 *P. falciparum*-naïve adults from North America, were removed from further analysis. The 655 reactive *P. falciparum* antigens meeting inclusion criteria for further analysis are listed in Appendix B. Antibody response data were dichotomized, with antibody intensities at least two standard deviations above the mean intensity of *P. falciparum*-naïve controls considered to be reactive. For each participant, the breadth of the antibody response was calculated as the proportion of reactive responses against the 655 *P. falciparum* antigens included in the analysis. Additionally, the mean intensity of the antibody response against each of the 655 reactive antigens was calculated for each participant. Bonferroni-corrected Mann-Whitney Tests were used to compare mean breadth and intensity of response among participants stratified by days since last infection.

Identification and Evaluation of Responses Informative of Exposure. To evaluate how informative responses to each *P. falciparum* antigen were at estimating recent malaria exposure, both alone and in combination, we modeled the ability of these antibody responses to predict two different metrics of an individual's *P. falciparum* exposure: (i) days since last infection and (ii) incidence of malaria in the prior year. Separate models estimating each log transformed outcome were fit using the

SuperLearner algorithm (215–218), which was chosen to balance two requirements: 1) that relationships between antibody responses and *P. falciparum* exposure be allowed to reflect natural, possibly nonlinear relationships as closely as possible, thus best reflecting the information present in antibody responses, and 2) that the entire process was fully automated, specifying the modelling procedure *a priori* to allow for cross validation and avoiding introduction of bias created by manually choosing the best fitting model procedure. In addition to antibody responses to the 655 reactive antigens, microscopy at the time of sample collection was included as a covariate in models predicting log days since last infection and age of participants was included as a covariate in models predicting log malaria incidence in the last year.

The SuperLearner is a flexible, data-adaptive ensembling approach that minimizes assumptions by returning a final model that is a convex combination of a potentially large number of candidate models. Nested within this framework, top antibody responses were selected prior to model fitting. The number of antibody responses selected was pre-specified for each model: models that incremented between selecting 1 and 30 responses were fit to evaluate the additional information obtained by allowing responses to a larger number of antigens to be chosen. Because of the large number of predictors, we also created a screening algorithm to be used within the SuperLearner to reduce the number of predictors to a tractable number. Specifically, our screening algorithm chose responses to top antigens using hierarchical criteria: first, lasso regression (219) was used to identify 1/3 of the responses, chosen to work in combination to predict exposure; next, the remaining responses were selected by iteratively choosing the best response as ranked by variable importance measures from random forest regression (220), then choosing the best response as ranked by p-values from Spearman's correlations with exposure. After feature selection, the SuperLearner predicted exposure (either log days since last infection or log incidence in the prior year) for each individual using a weighted average of five models: conventional multiple regression with all selected covariates in the model, lasso regression, random forests, support vector machines (221), and neural networks (222). Weights for each component model in the SuperLearner were calculated by non-negative least squares regression, minimizing the cross validated risk of the final estimator (216, 218).

To produce unbiased estimates of prediction accuracy, the entire SuperLearner process—including feature selection and model fitting—was cross validated using the CV.SuperLearner procedure. 20-fold cross validation was used, in which the data set was divided into 20 mutually exclusive subsets of as nearly equal size as possible. 19 subsets were then used for training the estimators, with predictions made on the hold-out validation set used to assess the performance of these estimators. This process of subsetting the data into different training and validation sets was repeated a total of 20 times to produce cross validated predictions of the exposure metric for each individual. Note that since the feature selection procedure was performed 20 different times as part of each cross validation process, it is possible that different subsets of individuals had models fit using responses to different antigens selected as covariates. SuperLearner cross validated predictions of exposure for each individual were used as the primary means by which we evaluated prediction accuracy and comprise all predictions in the results unless otherwise noted (e.g. where illustrating the prediction accuracy of a specific antibody response). R^2 values to evaluate prediction accuracy for each of the

two exposure metrics in individuals was calculated as: $R^2 = 1 - \frac{\sum_{i-1}^{N}(y_i - \hat{y}_i)^2}{\sum_{i-1}^{N}(y_i - \bar{y})^2}$, where y_i represents the actual value of the exposure metric (days since infection or incidence of malaria) for individual i and $\hat{y}_i$ represents the cross validated prediction for that individual.

Receiver Operator Characteristic (ROC) Curves (223) were used to evaluate the performance of models for classifying whether or not an individual was infected within a given time period. Correlations between entomologic data and actual or predicted incidence were determined using Spearman's rank correlation. After stratifying Kanungu participants' households by elevation, Mann-Whitney Tests were used to compare mean female Anophelene catches, incidence, and incidence predictions from serology in households at low versus high elevation.

To estimate the precision and accuracy of community estimates of exposure at various sample sizes, 5000 representative communities were created by subsampling actual study participants. Each individual participant had a measured incidence and a cross validated prediction of incidence generated from a SuperLearner model using 6 antibody responses, as described above. Participants were sampled with replacement, such that true individual incidences within the simulated community followed a Poisson distribution and the simulated communities had a range of population mean malaria incidences (0.5 to 4.0 episodes per person year). Mean predicted incidence for each simulated community was calculated by averaging incidence predictions for each individual within a simulated community.

Previously published clinical and microarray data from Malian participants (135) were used to evaluate the performance of selected responses in a different study population. Only plasma samples from participants aged 2-7 years, collected at the end of the 6-month malaria season, were included in this secondary analysis. Participants were followed for approximately 8 months before sample collection. Microarray data were normalized as previously described (135), and linear models were utilized to evaluate ability of responses selected in Ugandan participants to estimate exposure in the Malian population.

RESULTS

Study Populations and Clinical Outcomes. Participants consisted of 186 children from cohort studies in two districts of Uganda: Kanungu, where transmission is moderate (annual *E*ntomological Inoculation Rate (aEIR)=27), and Tororo, where transmission is intense (aEIR=125) (208). All participants were followed via active and passive surveillance for at least one year prior to the collection of the plasma samples analyzed here, allowing evaluation of recent exposure. Consistent with the higher intensity of *P. falciparum* exposure in Tororo, 95% of participants from this site were infected with *P. falciparum* in the last year, while only 64% of Kanungu participants had an infection detected (Table 2.1). Tororo participants had, on average, a higher incidence of malaria in the last year (median 7.8 episodes per person year) than participants from Kanungu (median 1.1 episode per person year). Similarly, among participants who had a *P. falciparum* infection detected in the previous year, participants

from Tororo were more recently infected with *P. falciparum* (median 28 days prior to the date of plasma collection) than those living in Kanungu (median 264 days).

***P. falciparum*-Specific Antibody Profiles Showed Decreased Responses with Increased Days Since Infection.** Out of 856 *P. falciparum* antigen probes on the microarray, 655 met the minimal antibody reactivity criteria for inclusion (Appendix B). Recent infection with *P. falciparum* was associated with greater breadth and intensity of response ($p<0.001$ for Spearman's correlations between both breadth ($r=-0.72$) and intensity ($r=-0.52$) and days since last infection). Notably, the overall breadth and intensity of anti-*P. falciparum* antibody responses was comparable between the two sites amongst participants whose days since last *P. falciparum* infection were similar (Figure 2.1). As such, data from both sites were combined for all subsequent analyses. Visualization of individual participants' antibody profiles across sites showed increased antibody reactivity in participants who were more recently infected (Figure 2.2). Linear regression demonstrated that mean antibody response decreased significantly over time following *P. falciparum* infection ($R^2=0.23$, $p<0.001$), consistent with published findings (12, 127, 205, 224–229).

Antibody Responses Most Predictive of an Individual's Exposure to *P. falciparum*. To identify antibody responses to *P. falciparum* antigens that were most informative of an individual's recent exposure to *P. falciparum*, a flexible prediction method that made few assumptions about the nature of the relationship between *P. falciparum* exposure and antibody intensity (details in Methods) was used to algorithmically identify top candidate antigens and model the ability of responses to the selected antigens to predict exposure. Our goal was to identify *P. falciparum* antigens inducing antibody responses that, in combination, produced the best predictions of an individual's (i) days since last *P. falciparum* infection and (ii) incidence of symptomatic malaria in the last year. To ensure unbiased estimates of prediction accuracy, the entire process—including antibody selection and model selection and fitting—was automated and cross validated. In other words, for every participant, predicted exposure was calculated based on choosing antibody responses and fitting their relationship to exposure in *other* participants, resulting in a conservative estimate of prediction accuracy.

While the microarray approach used in this study allowed us to screen responses to a large number of *P. falciparum* antigens, ideally information regarding exposure could be generated from responses to a limited number of antigens. To determine the trade-off between the number of responses measured and the accuracy in predicting an individual's prior exposure, we evaluated R^2 values when allowing the model to select between 1 to 30 antibody responses, measured as either continuous (antibody intensity) or binary (reactive versus non-reactive) variables (Figure 2.3). Since microscopy is often performed during cross-sectional surveys, we evaluated the information serology provided in addition to whether or not the participant had parasites detectable by microscopy at the time of sampling. Microscopy together with continuous antibody responses to a single selected antigen explained more than 60% of the variance in predicting days since an individual was last infected, while data from microscopy alone explained only 20% of the variance (Figure 2.3). Microscopy together with continuous

responses to 3 antigens explained 66% of the variance, and only marginal improvements in prediction accuracy were obtained when responses to additional antigens were included. When dichotomized, responses to a single antigen provided less information than those based on continuous measurements, but this loss of information could be compensated for by adding additional antigens; prediction accuracy was similar for continuous and binary data once responses to at least 20 antigens were included. Randomly selected dichotomized antibody responses also provided information but not nearly as efficiently, with continuous responses to 1 algorithmically selected antigen providing more accurate predictions than binary responses to 30 randomly selected antigens. Predicting the incidence of symptomatic malaria in the prior year using data on participants' age in combination with responses to increasing numbers of *P. falciparum* antigens produced analogous results, though binary and continuous responses to selected antigens provided similar information, and maximum accuracy was not reached until 6 responses were included. Together, these data indicate that accurate predictions of an individual's recent exposure to malaria can be obtained from measuring antibody responses to a small number of selected antigens.

Responses to the top antigens selected for predicting both exposure metrics in individuals were generally high and followed the expected trend ($p<0.001$ for Spearman's correlation between both exposure metrics and mean intensity of responses to the top 10 antigens) of increased intensity in participants with higher exposure (Figures 2.4 – 2.6). Six of the top ten responses identified as most predictive of days since last infection were also identified as highly predictive of malaria incidence (Table 2.2), which is not surprising given that these two metrics of exposure are closely related. Of note, amongst responses commonly used in the past to evaluate an individual's exposure (AMA1, MSP1, MSP2, and CSP) (24, 104, 114, 190, 229, 230), none were within the top 10 responses predictive of days since infection in this setting, and only MSP2 and CSP were within the top 10 predictive of malaria incidence in our participants. Additional characteristics of proteins targeted by the most informative antibody responses are provided in Supplemental Tables S2.1 – S2.6.

Accuracy of Selected Responses in Predicting Exposure in Specific Cases. One valuable way a serologic assay could be used by a malaria control program would be to determine whether individuals had been infected with *P. falciparum* in the recent past. To investigate the ability of selected antibody responses to correctly classify an individual as being infected within the last 30, 90, or 365 days, we compared each individual's actual infection status in that time frame to cross validated predictions of days since infection using microscopy and continuous responses to 3 antigens. ROC curves demonstrated that measuring these responses accurately classified recent infection status for all 3 time frames (Figure 2.7, cross validated area under the ROC curve ranged from 0.86-0.93). Since selection of responses was cross validated, the responses measured in each participant were selected in a different group of individuals and there was some stochastic variation in which 3 responses were used for predictions in any given participant. This variation allowed for unbiased and generalizable estimation of prediction accuracy, and selection closely followed the ranking in Table 2.2. Similar results were obtained when linear models that included microscopy and

responses to the top 3 antigens (PF3D7_1002000, PF3D7_0402400, and PF3D7_1106300) as predictors were used to estimate days since an individual was last infected (Figure 2.7).

Accurate predictions of recent exposure for multiple individuals obtained from serology can be aggregated to estimate average recent exposure in a community. Taking the average of cross validated predictions of malaria incidence for each individual from Kanungu or Tororo, which were obtained by measuring continuous responses to 6 antigens at a single time point, was an excellent indicator of observed incidence in the last year at that site (predicted versus actual cases per person year was 1.1 (95% CI=0.9-1.3) versus 1.5 (95% CI=1.1-1.8) in Kanungu and 5.4 (95% CI=4.8-5.9) versus 7.1 (95% CI=6.1-8.0) in Tororo). To extrapolate the potential for serology to estimate malaria incidence by testing small numbers of participants from communities with varying exposure, actual participants were sampled to represent testing 20 or 100 individuals from a community with a mean observed incidence ranging from 0.5 to 4.0 cases per person year. Mean predicted incidence was calculated using serology data from the same individuals in each sampled community. These simulations indicated that measuring antibody responses to a few antigens in a small subset of a community has the potential to provide accurate data on exposure (Figure 2.8).

A third use of individual-level estimates of exposure obtained from a serologic assay would be to identify heterogeneity in recent exposure within a community. Household GPS coordinates were collected for each participant in Kanungu, and elevation data for each household was used as a proxy for malaria exposure. In Kanungu, elevation was significantly correlated with the substantial variation in monthly household female Anophelene catches ($p<0.001$ for Spearman's correlation), with households at lower elevations in the north of the district having higher numbers of mosquitoes driving malaria exposure (Figure 2.9). Mean mosquito counts were significantly higher in the 36 households below 1100m, as compared to the 35 houses above this elevation (7.8 versus 2.0; $p<0.001$ for Mann-Whitney Test). Each individual's malaria incidence in the last year—as measured by passive surveillance—followed a spatial pattern that was also significantly correlated with household elevation ($p<0.001$ for Spearman's correlation), with measured incidence significantly higher amongst participants living in houses below versus above 1100m (2.2 versus 0.8 cases per person year; $p=0.001$ for Mann-Whitney Test). Similarly, predicted incidence in the last year—based on responses to 6 antigens from a single cross-sectional sample from each individual—correlated well with household elevation ($p<0.001$ for Spearman's correlation) and was able to detect spatial heterogeneity in exposure, predicting 1.4 versus 0.9 cases per person year in houses below and above 1100m, respectively ($p=0.005$ for Mann-Whitney Test). Predictions of incidence based on serology correlated more tightly with elevation than observed incidence measurements, possibly because an individual's incidence of malaria is confounded by the interplay between exposure and immunity (Figure 2.9B). Although accurate fine-scale assessments of spatial heterogeneity require denser sampling than was carried out here, these results indicate that individual incidence predictions from serology may be used to map out the heterogeneous distribution of malaria across a community.

Performance of Selected Antigens in a Different Population. We utilized previously published array data from 94 Malian children (135), aged 2-7 years, to determine whether the antigens selected in our Ugandan cohorts could also estimate an individual's exposure to malaria in a different population. Of note, this separate validation set represents individuals with different genetic backgrounds living in a different epidemiologic setting comprised of highly seasonal malaria exposure. Only plasma samples collected at the end of the 6-month malaria season were included in the analysis. The Mali array contained 4 out of the top 10 antigens (PF3D7_0711700, PF3D7_0800300, PF3D7_0501101.1, and PF3D7_0731600) inducing responses best able to predict days since last infection in Ugandan children. These 4 antigens were also highly immunogenic in Malian children (Figure 2.10A). Responses to these antigens were more closely associated with days since infection than overall responses ($r=-0.39$, $p<0.001$ versus $r=-0.24$, $p=0.02$ for Spearman's correlations between days since infection and mean response to top versus all antigens), similar to what was seen in Ugandan children and providing further support for the generalizability of our approach. Estimated days since last *P. falciparum* infection obtained from linear models using microscopy data and responses to one antigen (PF3D7_0711700) as predictors were able to classify an individual as being infected within the last 30 or 90 days (Figure 2.10B). Note that since 96% of this cohort was infected in the prior year, we did not classify individuals by whether they were infected within the last 365 days.

DISCUSSION

With the limited resources available for malaria control and elimination, it is imperative to be able to accurately and efficiently evaluate malaria exposure in different communities so that these resources can be used carefully and in a targeted way. In this study we demonstrate the utility of an innovative approach to identify a number of promising and novel serologic biomarkers of recent *P. falciparum* exposure. Detailed individual histories of *P. falciparum* infection obtained from cohort participants were used to select the most informative antibody responses to hundreds of antigen candidates using data-adaptive statistical models. Our results, confirmed through rigorous cross validation, demonstrate that accurate predictions of an individual's exposure history can be produced by measuring antibody responses to just a few *P. falciparum* antigens selected using this approach. Evaluation in multiple scenarios suggests that these serologic data are capable of providing precise and accurate estimates of exposure for individuals and communities.

Serologic surveys have been used to estimate *P. falciparum* exposure for over 40 years. With the push towards elimination and the advent of standardized assays and analytical approaches such as evaluating rates of seroconversion to specific *P. falciparum* antigens, serology has recently become more attractive as an epidemiologic tool (110–112, 175, 189, 191–201, 231, 232). The antigenic targets for such assays have been mostly limited to a small set of readily available recombinant proteins, generally selected for recognition by high titers of antibodies in immune individuals and not for their ability to provide quantitative information regarding exposure (233, 234). Recent studies have evaluated responses to multiple antigens simultaneously,

suggesting that certain responses may be more informative of exposure in particular settings (127, 235, 236). A fundamental distinction between prior efforts and the approach taken in this study is that here recent *P. falciparum* exposure in individuals was used to identify the most informative antibody responses amongst hundreds of candidates. This approach allowed for accurate, quantitative calibration of the relationships between identified responses and independent measures of exposure in individuals, in contrast to the coarser population-level relationships established by existing serology assays. The power of obtaining these individual-level estimates is illustrated by their ability to accurately identify individuals with infection in the recent past, with "recent" defined by relevant thresholds spanning one year; to obtain precise estimates of malaria incidence in a community from cross-sectional samples from as few as 20 individuals; and to accurately estimate heterogeneity in recent exposure within a community using data from a single time point.

A commonly perceived limitation of the protein array platform used in this study is the use of an *E. coli*-based cell-free expression system, in which some conformational epitopes may not be presented due to improper protein folding. While this limitation may have resulted in the lack of identification of some potential biomarkers, this concern is largely mitigated by the ability to screen hundreds of responses simultaneously, many of which likely provide similar information. Indeed, the identification of antigen targets which may be subsequently easier to produce is of potential benefit. Furthermore, while some potential biomarkers may have been missed, our results suggest that increasing the number of antigens beyond a few good candidates may be unnecessary. Of note, the array used in this study also included purified, validated recombinant proteins for commonly used antigens such as AMA1 and MSP1 at a wide range of dilutions, and none of these constructs were identified as amongst the most informative in our study.

Importantly, while the approach for discovery outlined here should be generalizable to a broad range of exposure settings and age ranges, the specific serologic biomarkers identified may not be as useful in other exposure contexts. Further evaluation is needed to assess whether the serologic biomarkers identified here are cross-reactive with other *Plasmodium* species (118), and whether the genetic background of parasites in certain areas may affect responses to selected antigens, which are currently based on the *P. falciparum* 3D7 strain reference sequence (237). Interestingly, amongst the top 38 antigens predictive of exposure, 7 map to PfEMP1 proteins, and at least 5 more are predicted to be exported (Figures 2.5B and 2.6B), indicating that genetic variation between *P. falciparum* strains may be important factor to consider. However, consistent with the previously published finding that intracellular domains of PfEMP1 proteins are more highly recognized by antibodies than hypervariable extracellular fragments (156), all 7 PfEMP1 peptide fragments selected in our cohorts correspond to highly conserved intracellular domains. It will be of interest in future studies to evaluate the relative contribution of measuring responses to different variants of particular antigens in evaluating exposure (237).

Additionally, dynamics of antibody acquisition and maintenance vary based on exposure intensity and age, thus the degree to which some serologic biomarkers predict exposure will likely vary in these contexts (110, 205, 235, 238). Our study only evaluated participants aged 3 to 7 years living in two areas of Uganda with moderate or high exposure, and aged 2 to 7 in an area with intense seasonal exposure in Mali. The

P. falciparum biomarkers identified here are likely to be of value in similar settings but require further validation in these and other settings and age groups. In particular, assessment of serologic responses in communities before and after implementation of malaria control measures will be needed to validate the utility of responses in evaluating the impact of these interventions. Replicating the approach outlined here in a broader array of epidemiologic settings will provide an efficient means of identifying a set or sets of biomarkers appropriate across these contexts.

If the validation approaches above can be confirmed, additional steps are then needed to translate the novel biomarkers identified here and in future studies into high throughput serologic assays for routine surveillance (118). Detailed characterization of the kinetics of identified responses within individuals over time will enable development of more tailored and precise statistical models to estimate recent exposure, in contrast to the more flexible techniques used here for biomarker identification (239–242). Target antigens will need to be expressed as standardized, purified reagents to allow for consistent measurement of responses. Finally, simple, inexpensive assay platforms, e.g. based on ELISA or portable lateral flow devices, will need to be optimized to allow serologic assessments of malaria exposure to be performed on field samples such as dried blood spots or whole blood obtained from finger-pricks in appropriate settings. Simple assays derived from such an approach have the potential to generate rich epidemiologic surveillance data that would be widely accessible to malaria control programs.

The potential to obtain more accurate estimates of *P. falciparum* exposure from small sample sizes makes further attractive the already promising use of serology as a key malaria surveillance tool. In areas requiring wide-scale malaria control, collecting national serologic data, e.g. as part of a malaria indicator survey, could improve targeting of control interventions to broad areas with the highest exposure risk. In areas of lower exposure, focal surveys could allow interventions targeted to smaller-scale hotspots at the level of villages or groups of households (230, 243). In settings nearing malaria elimination, serology could identify individuals infected in the recent past, allowing identification of spatial or demographic risk factors and ultimately certifying that elimination has occurred (244, 245). Finally, repeated evaluation of recent exposure over time could be used to assess the impact of control interventions in reducing exposure and detect re-introduction of *P. falciparum* transmission after local elimination.

In addition to their utility in surveillance, serologic estimates of exposure can provide a valuable research tool. As it is difficult to measure protection against malaria without knowing the underlying rate of infection, studies of naturally acquired or vaccine-induced immunity are confounded by heterogeneous *P. falciparum* exposure (217, 246–249). The ability to estimate an individual's recent exposure at the beginning of a study and/or rates of infection during follow-up would be useful in assessing protection. To be valuable in this context, consideration would need to be taken to identify biomarkers of exposure not directly involved in mediating immune protection or strongly influenced by blood stage immunity. Additionally, serologic outcomes may provide a cost-effective means for measuring the effect of new interventions on a study population, especially when it is not practical to perform detailed, continuous clinical or parasitological surveillance of all participants. Given the broad utility of serology, identifying the

serologic biomarkers that provide the most accurate estimates of exposure seems a worthwhile investment.

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Table 2.1. Descriptive statistics of the study sites and participants.

	Kanungu	Tororo
Number of participants	107	79
Median age in years (range)	5.2 (3.1-6.8)	4.0 (3.9-4.4)
Female gender	51%	42%
Median monthly female *Anopheles* per house, n (range)	2 (0-29)	n/a
Median malaria incidence in the last year, ppy (range)	1.1 (0-8.5)	7.8 (0-19.0)
Parasitemic at time of sample collection, n (%)	9 (8%)	13 (16%)
Median *P. falciparum* density at sample collection*, (range)	$1x10^4$ ($64-7x10^4$)	$5x10^4$ ($3x10^3-3x10^5$)
Participants having ≥1 infection in the last 30 days, n (%)	20 (19%)	43 (54%)
Participants having ≥1 infection in the last 90 days, n (%)	37 (35%)	64 (81%)
Participants having ≥1 infection in the last 365 days, n (%)	69 (64%)	75 (95%)
Median days since last *P. falciparum* infection**, (range)	264 (0-340)	28 (0-332)

*Only participants who were parasitemic at the time of sample collection were included.

**Only participants who had at least one *P. falciparum* infection recorded in the previous year were included.

Table 2. Most informative serologic markers of malaria exposure.

Rank	Gene ID	Description
Antigens Predicting Days Since an Individual was Last Infected:		
1	PF3D7_1002000*	Plasmodium exported protein, hyp2
2	PF3D7_0402400	Plasmodium exported protein, GEXP18
3	PF3D7_1106300*	exonuclease, putative
4	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1
5	PF3D7_0800300*	erythrocyte membrane protein 1, PfEMP1
6	PF3D7_0501100.1	heat shock protein 40, type II, HSP40
7	PF3D7_0423700*	early transcribed membrane protein 4, ETRAMP4
8	PF3D7_1020800*	dihydrolipoamide acyltransferase component E2, DLAT
9	PF3D7_0731600*	acyl-CoA synthetase, ACS5
10	PF3D7_1002100	PF70 protein, PF70
Antigens Predicting an Individual's Malaria Incidence in the Last Year:		
1	PF3D7_1002000*	Plasmodium exported protein, hyp2
2	PF3D7_1020800*	dihydrolipoamide acyltransferase component E2, DLAT
3	PF3D7_0731600*	acyl-CoA synthetase, ACS5
4	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5
5	PF3D7_0801000	Plasmodium exported protein, PHISTc
6	PF3D7_0304600	circumsporozoite protein, CSP
7	PF3D7_0206800	merozoite surface protein 2, MSP2
8	PF3D7_0800300*	erythrocyte membrane protein 1, PfEMP1
9	PF3D7_1106300*	exonuclease, putative
10	PF3D7_0423700*	early transcribed membrane protein 4, ETRAMP4

*Antigen was within the top 10 for predicting both days since last *P. falciparum* infection and malaria incidence in the last year.

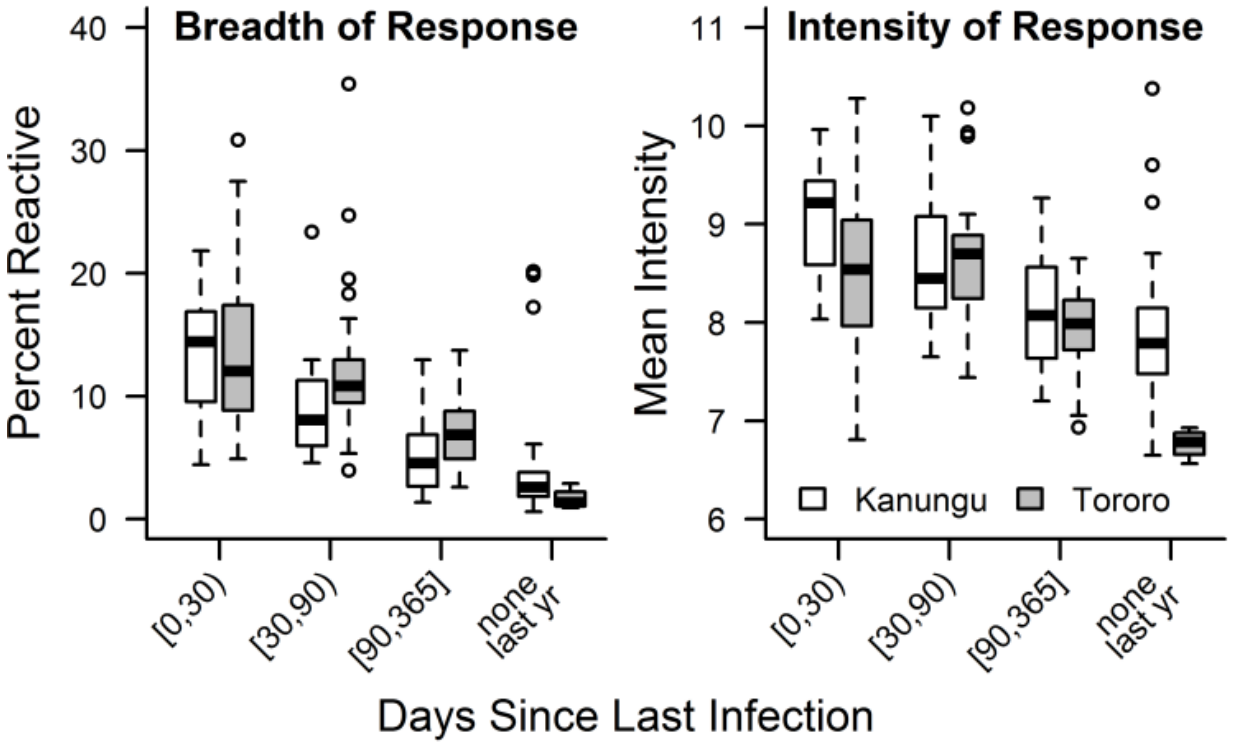


Figure 2.1. Breadth and intensity of antibody responses decrease with days since infection. Breadth of response for each participant was calculated as the percentage of antibody responses that were reactive (at least 2 standard deviations above malaria naïve controls) to the 655 included *P. falciparum* antigens. Mean intensity for each participant was calculated from normalized intensities of antibody responses. Breadth of responses did not significantly differ between participants from the two sites once stratified by days since infection, though participants from Tororo were on average more recently infected. Intensity of responses also did not differ, except for participants who were not infected with *P. falciparum* within the last year (p=0.008, Mann-Whitney Test with Bonferroni correction).

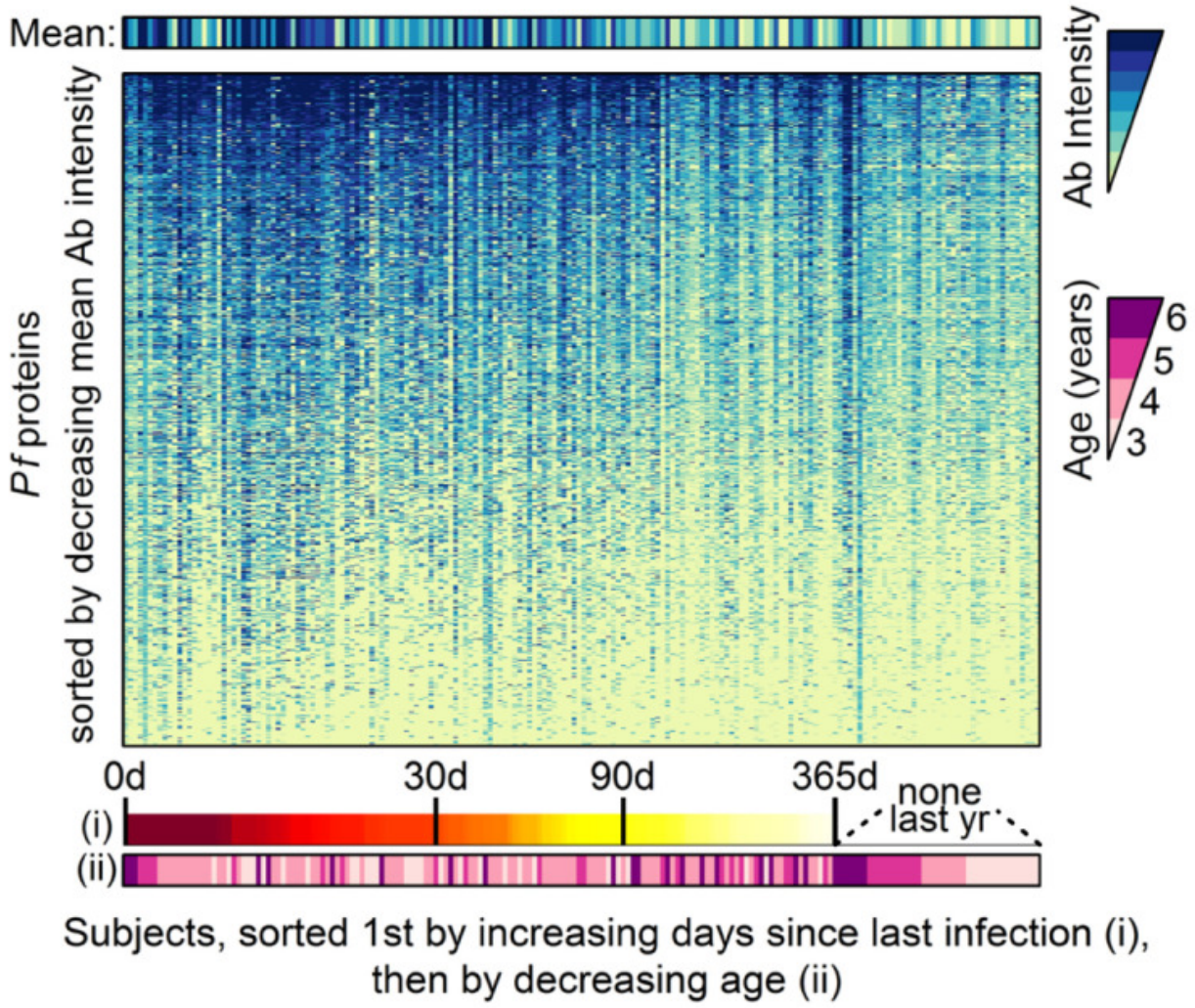


Figure 2.2. Heat maps of intensities of antibody responses to all 655 reactive *P. falciparum* antigens demonstrate that responses are generally higher in participants who were most recently infected. Among participants who did not have an infection detected in the year prior to sample collection, the oldest participants had higher overall responses.

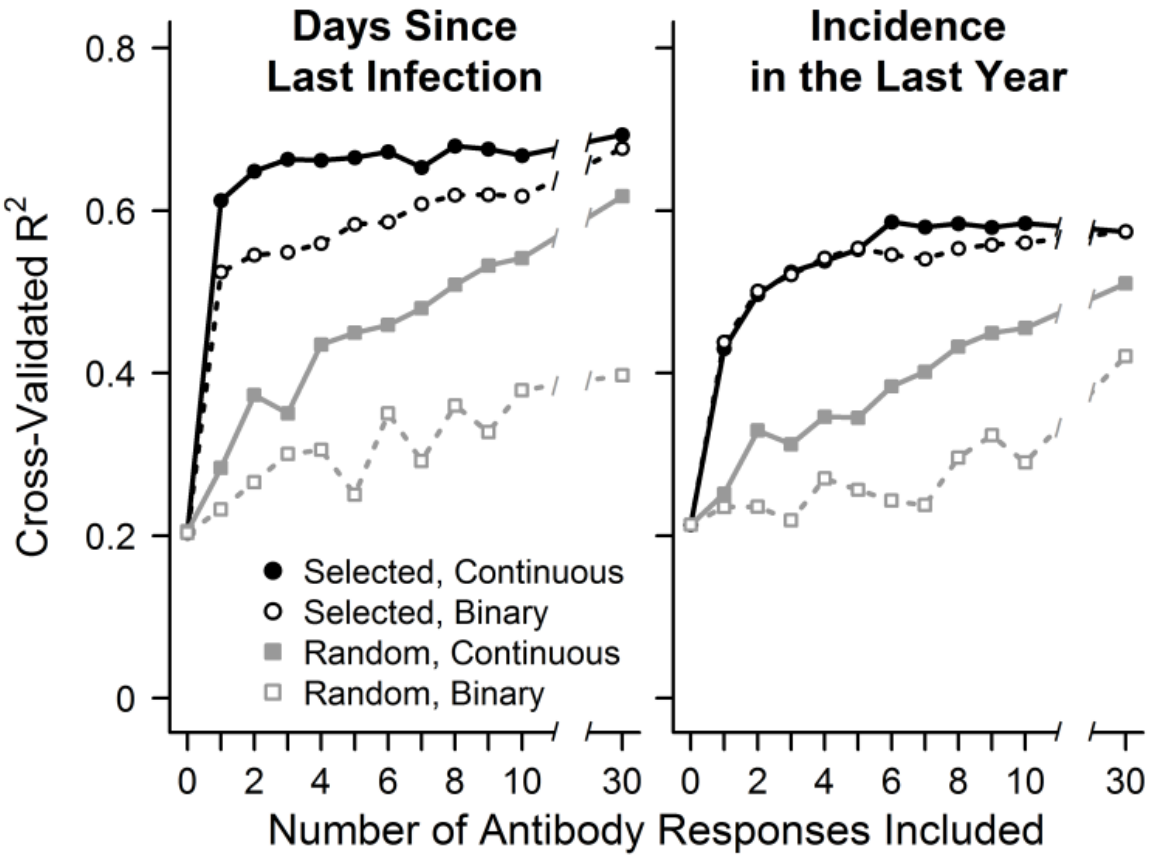


Figure 2.3. Relationship between prediction accuracy and antibody responses to the number of *P. falciparum* antigens included in the prediction model. The y-axis indicates accuracy of cross validated predictions, measured as R^2, or percentage of variance explained in the outcome being predicted. Models predicting days since last infection (left) utilized baseline microscopy results and antibody response data to 0 (microscopy only) to 30 antigens. Models predicting malaria incidence in the last year (right) utilized each participant's age and antibody response data to 0 (age only) to 30 antigens. Antibody responses selected based on their ability to predict the outcome in the training set ("Selected") performed better than antigens chosen at random ("Random"), despite selection and prediction being performed on independent sets of samples. Antibody responses evaluated as continuous variables performed better than binary responses for days since infection, but similarly for incidence predictions.

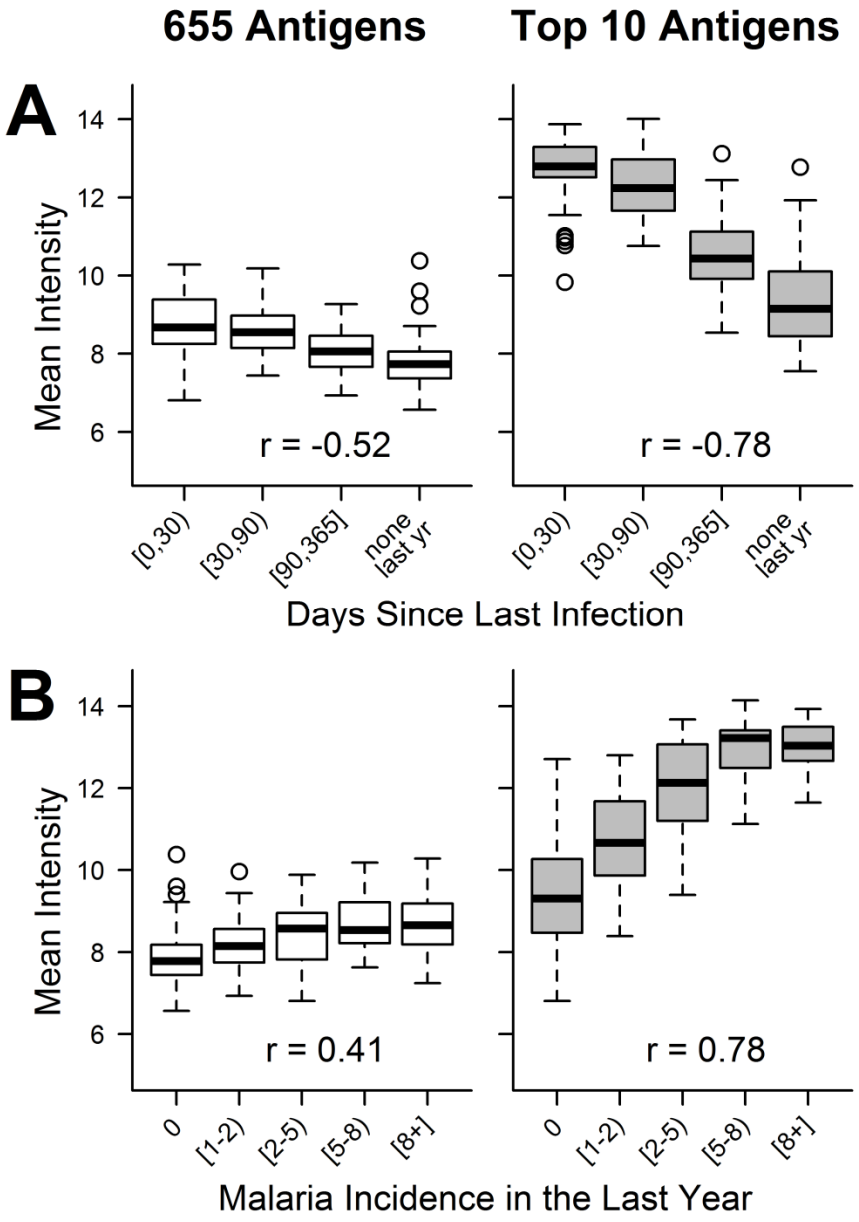
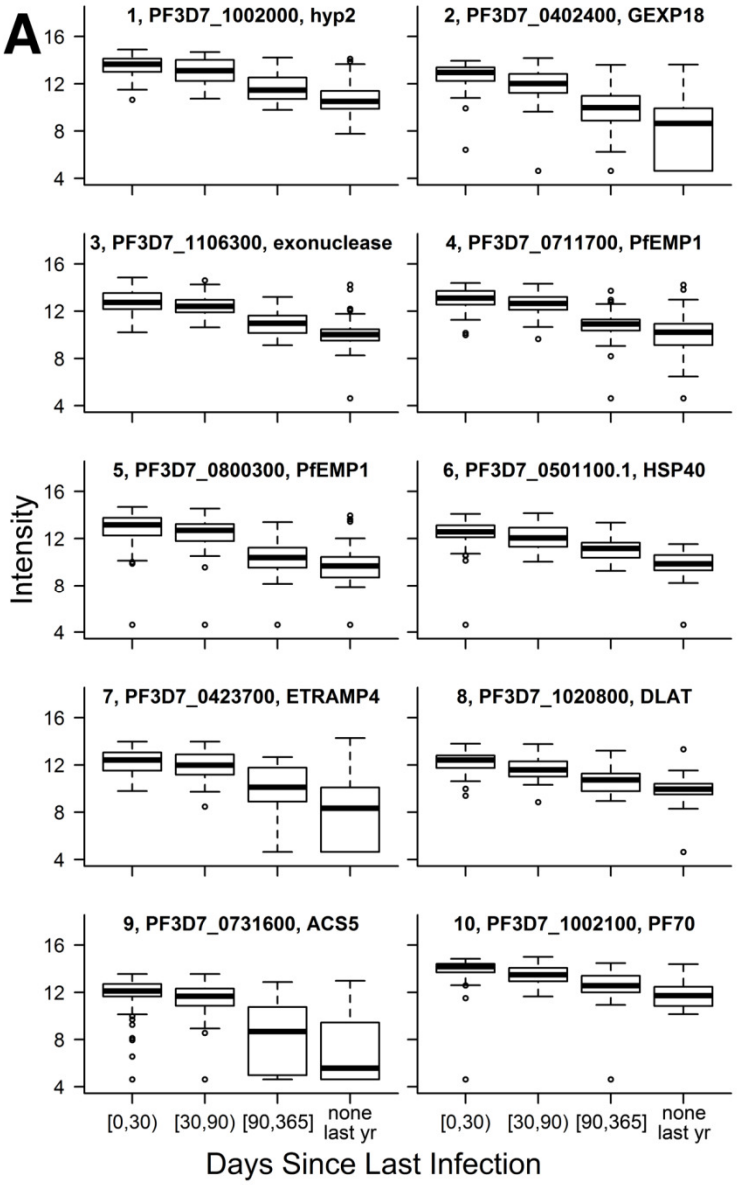


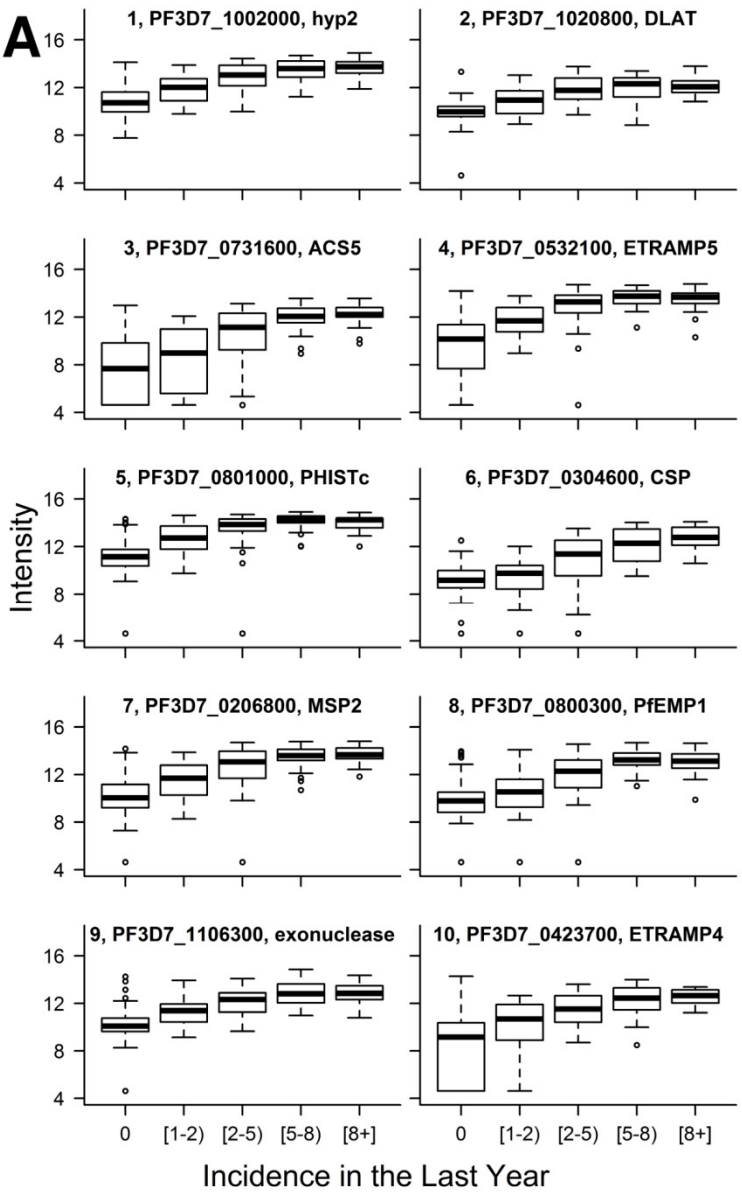
Figure 2.4. Mean intensity of antibody responses for participants grouped by exposure outcome: (A) Days since last infection (0 to <30d, n=59; 30 to <90d, n=42; 90 to 365d, n=43; no *P. falciparum* infection detected in the last year, n=42) and (B) malaria incidence in the last year (0, n=47; 1 to <2, n=39; 2 to <5, n=42; 5 to <8, n=34; ≥8, n=24). Responses against the top 10 antigens selected for each exposure metric (right) were highly immunogenic and demonstrated more consistent associations with exposure, by design, than overall responses (left). Spearman's *rho* for correlations between the exposure metric and an individual's mean antibody response to the set of antigens indicated are presented in each plot.



B

	Antigen	AUC Last 30d	AUC Last 90d	AUC Last 365d
1	PF3D7_1002000 (hyp2)	0.91	0.91	0.87
2	PF3D7_0402400 (GEXP18)	0.91	0.92	0.86
3	PF3D7_1106300 (exonuclease)	0.91	0.94	0.88
4	PF3D7_0711700 (PfEMP1)	0.91	0.92	0.84
5	PF3D7_0800300 (PfEMP1)	0.88	0.90	0.83
6	PF3D7_0501100.1 (HSP40)	0.87	0.90	0.92
7	PF3D7_0423700 (ETRAMP4)	0.89	0.91	0.88
8	PF3D7_1020800 (DLAT)	0.92	0.90	0.88
9	PF3D7_0731600 (ACS5)	0.88	0.90	0.84
10	PF3D7_1002100 (PF70)	0.92	0.90	0.89

Figure 2.5. (A) Intensity of antibody responses to the Top 10 *P. falciparum* antigens predicting days since last infection decrease over time. Each boxplot's title indicates "Rank, Gene ID, Annotation." (B) Days since a participant was last infected with *P. falciparum* were estimated from linear models that included microscopy and responses to one of these Top 10 antigens as covariates. Based on these predictions, receiver operating characteristic (ROC) curves classified whether individuals were infected within the last 30, 90, and 365 days. Areas under the curve (AUCs) were calculated for each ROC curve.



B

	Antigen	Predicted Mean Incidence (95% CI) Kanungu *Incidence:1.5*	Tororo *Incidence: 7.1*
1	PF3D7_1002000 (hyp2)	1.3 (1.1-1.6)	4.4 (4.0-4.9)
2	PF3D7_1020800 (DLAT)	1.4 (1.1-1.7)	4.1 (3.6-4.6)
3	PF3D7_0731600 (ACS5)	1.4 (1.2-1.6)	4.1 (3.7-4.5)
4	PF3D7_0532100 (ETRAMP5)	1.4 (1.2-1.6)	4.0 (3.7-4.3)
5	PF3D7_0801000 (PHISTc)	1.4 (1.2-1.6)	4.1 (3.7-4.5)
6	PF3D7_0304600 (CSP)	1.2 (1.0-1.3)	4.4 (4.0-4.9)
7	PF3D7_0206800 (MSP2)	1.2 (1.0-1.4)	4.3 (3.9-4.7)
8	PF3D7_0800300 (PfEMP1)	1.5 (1.3-1.7)	3.7 (3.3-4.1)
9	PF3D7_1106300 (exonuclease)	1.3 (1.1-1.6)	4.3 (3.8-4.8)
10	PF3D7_0423700 (ETRAMP4)	1.4 (1.2-1.6)	4.1 (3.7-4.5)

Figure 2.6. (A) Intensity of antibody responses to each of the Top 10 *P. falciparum* antigens predicting malaria incidence in the last year increase with increasing exposure. Each boxplot's title indicates "Rank, Gene ID, Annotation." (B) For each participant, incidence last year was estimated from linear models that included age and responses to one of these Top 10 antigens as covariates. Predicted incidences for each participant were averaged across subjects from each study site to calculate population mean incidences for Kanungu and Tororo. Observed population mean incidence was 1.5 cases per person-year in Kanungu and 7.1 cases per person-year in Tororo.

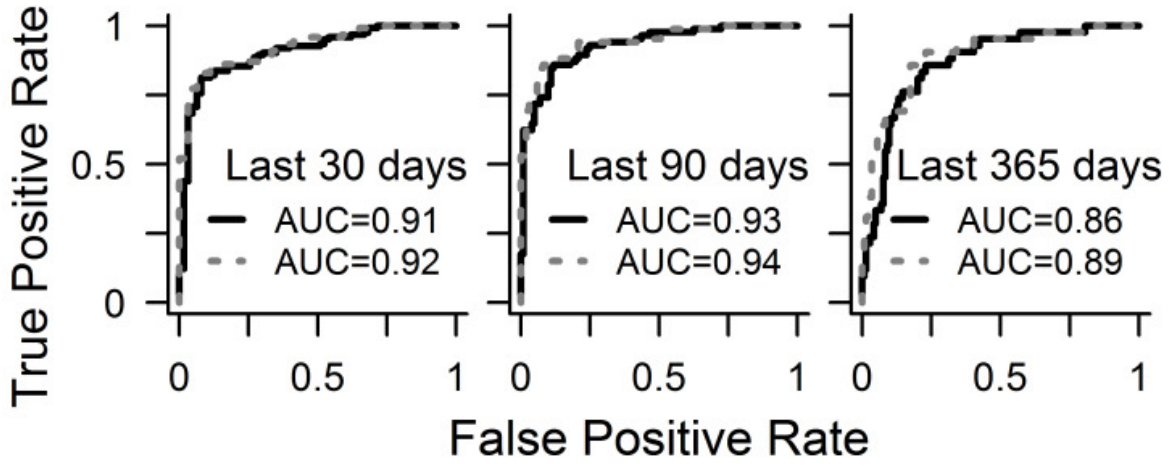


Figure 2.7. Receiver operating characteristic (ROC) curves for predictions of days since infection using responses to 3 antigens and microscopy data were able to accurately classify whether an individual was infected within the last 30, 90, or 365 days, as indicated by the high area under the curve (AUC) values. Solid lines represent SuperLearner predictions, in which both antigen selection and model fitting were cross validated; dashed lines represent predictions from linear models, in which the included antigens (PF3D7_1002000, PF3D7_0402400, and PF3D7_1106300) were preselected as the top 3 most predictive of days since last infection. An AUC of 0.5 indicates a classifier that performs no better than random, while an AUC of 1 indicates a perfect classifier.

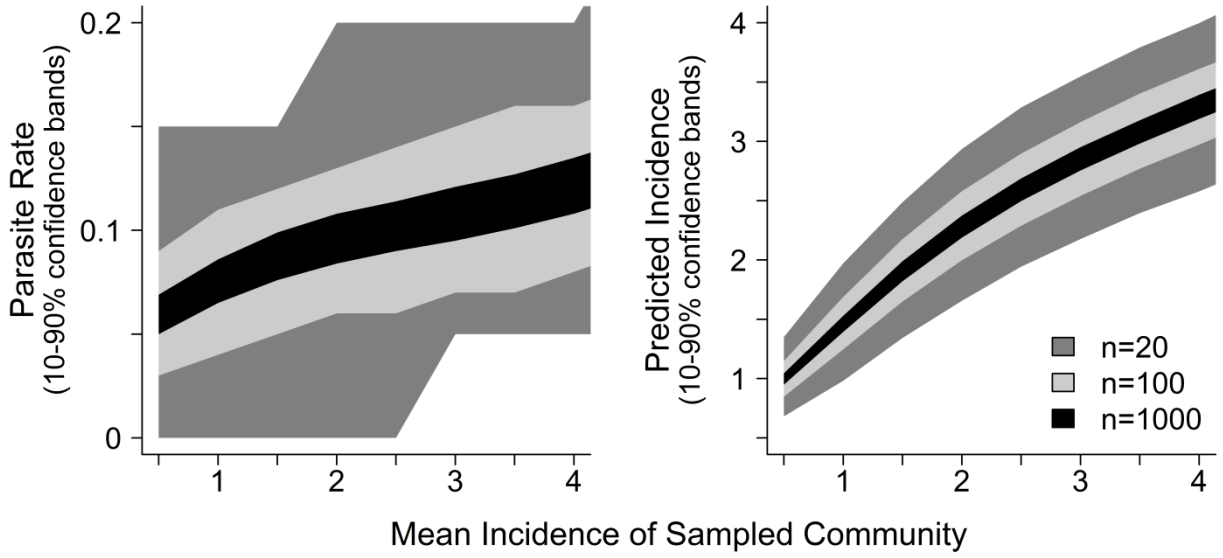


Figure 2.8. Representative communities with population mean malaria incidences ranging from 0.5 to 4.0 episodes per person year were created by subsampling (with replacement) 20,100, or 1000 actual study participants following Poisson distributions. Analyses of actual participant data for individuals assigned to each of these simulated communities was used to calculate parasite rates (left) and predictions of incidence using serology (right). Left: Parasite rates were determined by calculating the proportion of subjects in each simulated community who had parasites detected by microscopy. Accurate measures of a community's parasite rate were only obtained once a large number of individuals were sampled. Furthermore, there was not a strong correlation between increases in community-wide incidence and parasite rate. Right: Predicted community incidences were aggregated from cross validated estimates for each individual, which were produced using antibody responses to 6 antigens. Serologic analysis of a small number of individuals produced accurate predictions of mean incidence for the community.

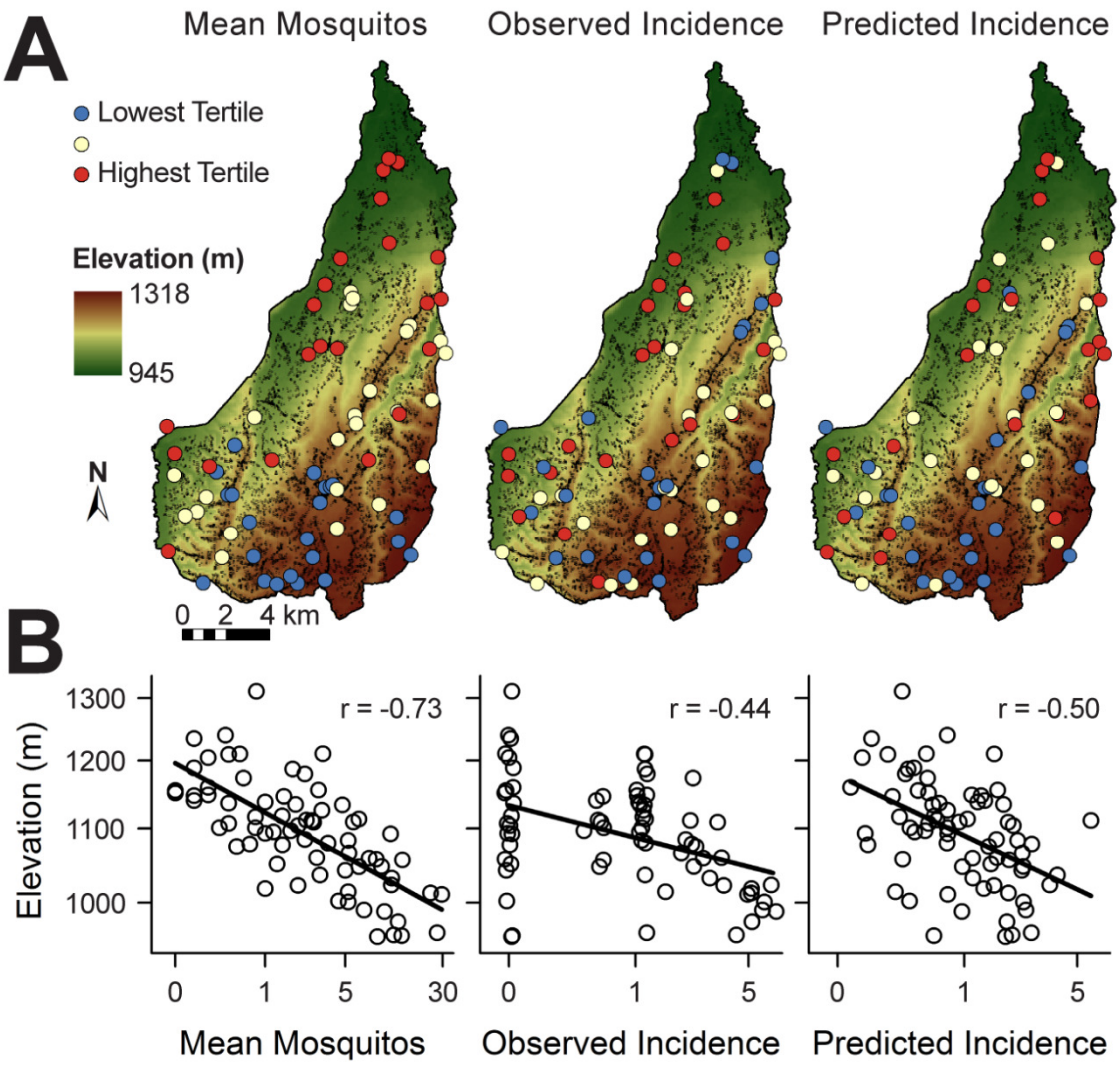


Figure 2.9. (A) Spatial heterogeneity in *P. falciparum* exposure is captured by serologic predictions of incidence. Average monthly counts of female Anopheline mosquitos (left), observed malaria incidence over one year (middle), and cross validated predictions of incidence using antibody responses to 6 antigens taken at a single time point (right) are plotted for each study household in Kanungu, Uganda, with colors indicating the tertile for each household. Some households contain more than one included study participant; in these cases, the household mean is plotted for observed and predicted incidence. Small black dots represent households that were not sampled. (B) Scatterplots of household elevation (a proxy for *P. falciparum* exposure) versus mean mosquito counts (left), observed (middle), or predicted (right) malaria incidence in the last year. All three metrics are significantly associated with elevation ($p<0.001$ for Spearman's correlation). Individuals residing in households at low elevations but having no episodes of clinical malaria in the last year are likely highly exposed and immune; serologic predictions of incidence suggests that these individuals were, in fact, exposed to *P. falciparum.*

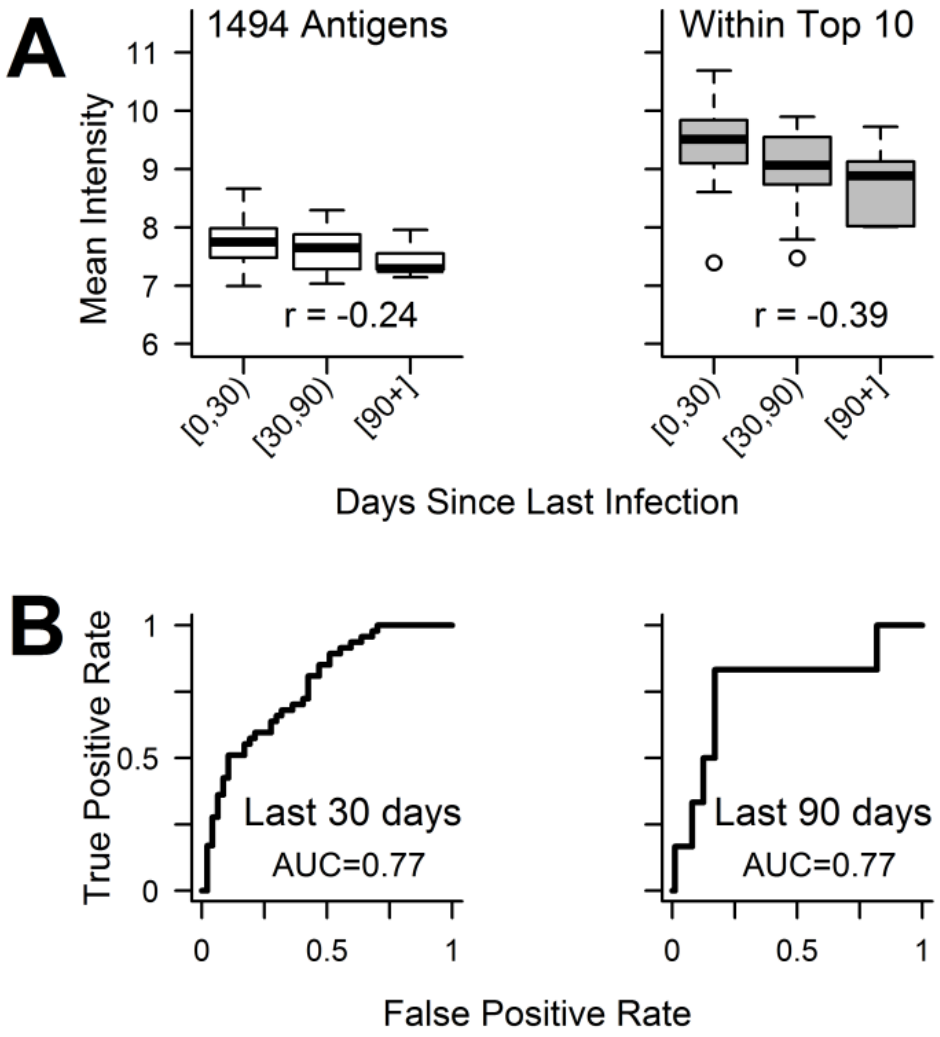


Figure 2.10. Plasma samples from Malian children were used to probe microarrays that contained 4 of the Top 10 antigens inducing responses in Ugandan children that were predictive of days since last infection. (A) Mean intensity of antibody responses in Malian participants grouped by days since last infection (0 to <30d, n=47; 30 to <90d, n=41; >90d, n=6). Responses to antigens within the Top 10 selected in Ugandans (right) were highly immunogenic in Malian participants and also demonstrated more consistent associations with exposure than overall responses (left). Spearman's *rho* for correlations between an individual's days since last *P. falciparum* infection and mean antibody response to the set of antigens indicated are presented in each plot. (B) Receiver operating characteristic (ROC) curves for predictions of days since infection from linear models using 1 antigen (PF3D7_0711700) and microscopy data were able to classify whether an individual from Mali was infected within the last 30 or 90 days.

Supplemental Table S2.1. Function of *P. falciparum* antigens selected for predicting days since last infection.

Rank	Gene ID	Description	Function
1	PF3D7_1002000	Plasmodium exported protein, hyp2	
2	PF3D7_0402400	Plasmodium exported protein, GEXP18	nucleotide binding
3	PF3D7_1106300	exonuclease, putative	exonuclease activity; nucleic acid binding
4	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
5	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
6	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	heat shock protein binding; unfolded protein binding
7	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4	
8	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT	dihydrolipoyllysine-residue acetyltransferase activity; pyruvate dehydrogenase activity
9	PF3D7_0731600	acyl-CoA synthetase, ACS5	AMP binding; long-chain fatty acid-CoA ligase activity; catalytic activity
10	PF3D7_1002100	PF70 protein, PF70	
Top 20	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1	
	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5	
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP	
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_0801000	Plasmodium exported protein, PHISTc	
	PF3D7_0936300	ring-exported protein 3, REX3	
	PF3D7_1033200	early transcribed membrane protein 10.2, ETRAMP10.2	
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1	GTPase activator activity; protein binding
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_1353100	Plasmodium exported protein	
Top 30	PF3D7_0304600	circumsporozoite protein, CSP	
	PF3D7_0414700	GTP binding protein, putative	GTP binding; ferrous iron transmembrane transporter activity
	PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_0532400	Plasmodium exported protein, PHISTb	
	PF3D7_0620400	merozoite surface protein 10, MSP10	attachment of GPI anchor to protein
	PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_1024800	conserved Plasmodium protein	hydrolase activity
	PF3D7_1133400	apical membrane antigen 1, AMA1	host cell surface binding; protein binding
	PF3D7_1438100	secretory complex protein 62, SEC62	protein binding; protein transporter activity; intracellular signal transduction
	PF3D7_1477500	Plasmodium exported protein, PHISTb	

Supplemental Table S2.2. *P. falciparum* life cycle stage for expression of *P. falciparum* antigens selected for predicting days since last infection.

Rank	Gene ID	Description	Stage Expressed[1]				
			L	S	IE	M	G
1	PF3D7_1002000	Plasmodium exported protein, hyp2			X	X	
2	PF3D7_0402400	Plasmodium exported protein, GEXP18			X	X	X
3	PF3D7_1106300	exonuclease, putative					
4	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1		X			X
5	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1		X			
6	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	X		X	X	X
7	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4			X		X
8	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT	X				
9	PF3D7_0731600	acyl-CoA synthetase, ACS5	X	X	X	X	
10	PF3D7_1002100	PF70 protein, PF70			X		X
Top 20	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1					
	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5			X	X	
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP			X	X	X
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1		X	X	X	
	PF3D7_0801000	Plasmodium exported protein, PHISTc			X		X
	PF3D7_0936300	ring-exported protein 3, REX3			X		X
	PF3D7_1033200	early transcribed membrane protein 10.2, ETRAMP10.2			X	X	X
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1			X	X	X
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1		X		X	
	PF3D7_1353100	Plasmodium exported protein			X	X	X
Top 30	PF3D7_0304600	circumsporozoite protein, CSP		X			
	PF3D7_0414700	GTP binding protein, putative		X			
	PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1		X			
	PF3D7_0532400	Plasmodium exported protein, PHISTb			X	X	X
	PF3D7_0620400	merozoite surface protein 10, MSP10		X	X	X	
	PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1		X			
	PF3D7_1024800	conserved Plasmodium protein	X	X	X	X	X
	PF3D7_1133400	apical membrane antigen 1, AMA1		X	X	X	
	PF3D7_1438100	secretory complex protein 62, SEC62	X		X	X	X
	PF3D7_1477500	Plasmodium exported protein, PHISTb			X	X	

[1]Stage Expressed, by mass spec: Liver (L); sporozoite (S), intra-erythrocytic (IE); merozoite (M); gametocyte (G).

Supplemental Table S2.3. Localization in the *P. falciparum* parasite or human red blood cell for antigens selected for predicting days since last infection.

Rank	Gene ID	Description	Cellular Component[1]					
			HC	MC	CS	I	A	SCV
1	PF3D7_1002000	Plasmodium exported protein, hyp2			X	X		
2	PF3D7_0402400	Plasmodium exported protein, GEXP18				X		
3	PF3D7_1106300	exonuclease, putative				X	X	
4	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1	X		X			
5	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	X		X			
6	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	X					
7	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4			X			
8	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT			X		X	
9	PF3D7_0731600	acyl-CoA synthetase, ACS5						
10	PF3D7_1002100	PF70 protein, PF70			X			
Top 20	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1						
	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5						
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP			X			
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_0801000	Plasmodium exported protein, PHISTc				X	X	
	PF3D7_0936300	ring-exported protein 3, REX3						
	PF3D7_1033200	early transcribed membrane protein 10.2, ETRAMP10.2		X				X
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1				X		X
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_1353100	Plasmodium exported protein				X		
Top 30	PF3D7_0304600	circumsporozoite protein, CSP			X			
	PF3D7_0414700	GTP binding protein, putative			X		X	
	PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_0532400	Plasmodium exported protein, PHISTb						
	PF3D7_0620400	merozoite surface protein 10, MSP10			X			
	PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_1024800	conserved Plasmodium protein			X			
	PF3D7_1133400	apical membrane antigen 1, AMA1			X		X	
	PF3D7_1438100	secretory complex protein 62, SEC62			X	X		
	PF3D7_1477500	Plasmodium exported protein, PHISTb			X			

[1]Cellular Component: exported to host cell (HC); Maurer's cleft (MC); membrane/cell surface (CS); intracellular/cytoplasm/nucleus (I); apicoplast (A); symbiont-containing vacuole (SCV).

Supplemental Table S2.4. Function of *P. falciparum* antigens selected for predicting malaria incidence in the last year.

Rank	Gene ID	Description	Function
1	PF3D7_1002000	Plasmodium exported protein, hyp2	
2	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT	dihydrolipoyllysine-residue acetyltransferase activity; pyruvate dehydrogenase activity
3	PF3D7_0731600	acyl-CoA synthetase, ACS5	AMP binding; long-chain fatty acid-CoA ligase activity; catalytic activity
4	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5	
5	PF3D7_0801000	Plasmodium exported protein, PHISTc	
6	PF3D7_0304600	circumsporozoite protein, CSP	
7	PF3D7_0206800	merozoite surface protein 2, MSP2	attachment of GPI anchor to protein; cell adhesion
8	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
9	PF3D7_1106300	exonuclease, putative	exonuclease activity; nucleic acid binding
10	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4	
Top 20	PF3D7_0207000	merozoite surface protein 4, MSP4	
	PF3D7_0402400	Plasmodium exported protein, GEXP18	nucleotide binding
	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	heat shock protein binding; unfolded protein binding
	PF3D7_0532400	Plasmodium exported protein, PHISTb	
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP	
	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_0936300	ring-exported protein 3, REX3	
	PF3D7_1002100	PF70 protein, PF70	
	PF3D7_1036400	liver stage antigen 1, LSA1	
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1	GTPase activator activity; protein binding
Top 30	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1	
	PF3D7_0620400	merozoite surface protein 10, MSP10	
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_0930300	merozoite surface protein 1, MSP1	attachment of GPI anchor to protein; pathogenesis
	PF3D7_1024800	conserved Plasmodium protein	hydrolase activity
	PF3D7_1100800	Maurer's cleft two transmembrane protein, MC-2TM	translocation of peptides or proteins into host
	PF3D7_1133400	apical membrane antigen 1, AMA1	host cell surface binding; protein binding
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	cell adhesion molecule binding; receptor activity; antigenic variation
	PF3D7_1401400	early transcribed membrane protein 14.1, ETRAMP14	
	PF3D7_1410400	rhoptry-associated protein 1, RAP1	

Supplemental Table S2.5. *P. falciparum* life cycle stage for expression of *P. falciparum* antigens selected for predicting malaria incidence in the last year.

Rank	Gene ID	Description	Stage Expressed[1]				
			L	S	IE	M	G
1	PF3D7_1002000	Plasmodium exported protein, hyp2			X	X	
2	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT	X				
3	PF3D7_0731600	acyl-CoA synthetase, ACS5	X	X	X	X	
4	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5			X	X	
5	PF3D7_0801000	Plasmodium exported protein, PHISTc			X		X
6	PF3D7_0304600	circumsporozoite protein, CSP		X			
7	PF3D7_0206800	merozoite surface protein 2, MSP2			X	X	
8	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1		X			
9	PF3D7_1106300	exonuclease, putative					
10	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4			X		X
Top 20	PF3D7_0207000	merozoite surface protein 4, MSP4					
	PF3D7_0402400	Plasmodium exported protein, GEXP18			X	X	X
	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	X		X	X	X
	PF3D7_0532400	Plasmodium exported protein, PHISTb			X	X	X
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP			X	X	X
	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1		X			X
	PF3D7_0936300	ring-exported protein 3, REX3			X		X
	PF3D7_1002100	PF70 protein, PF70			X		X
	PF3D7_1036400	liver stage antigen 1, LSA1					
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1			X	X	X
Top 30	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1					
	PF3D7_0620400	merozoite surface protein 10, MSP10		X	X	X	
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1		X	X	X	
	PF3D7_0930300	merozoite surface protein 1, MSP1	X	X	X	X	X
	PF3D7_1024800	conserved Plasmodium protein	X	X	X	X	X
	PF3D7_1100800	Maurer's cleft two transmembrane protein, MC-2TM			X	X	
	PF3D7_1133400	apical membrane antigen 1, AMA1		X	X	X	
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1		X		X	
	PF3D7_1401400	early transcribed membrane protein 14.1, ETRAMP14		X	X	X	
	PF3D7_1410400	rhoptry-associated protein 1, RAP1	X	X	X	X	X

[1]Stage Expressed, by mass spec: Liver (L); sporozoite (S), intra-erythrocytic (IE); merozoite (M); gametocyte (G).

Supplemental Table S2.6. Localization in the *P. falciparum* parasite or human red blood cell for antigens selected for predicting malaria incidence in the last year.

Rank	Gene ID	Description	Cellular Component[1]					
			HC	MC	CS	I	A	SCV
1	PF3D7_1002000	Plasmodium exported protein, hyp2			X	X		
2	PF3D7_1020800	dihydrolipoamide acyltransferase component E2, DLAT			X		X	
3	PF3D7_0731600	acyl-CoA synthetase, ACS5						
4	PF3D7_0532100	early transcribed membrane protein 5, ETRAMP5						
5	PF3D7_0801000	Plasmodium exported protein, PHISTc				X	X	
6	PF3D7_0304600	circumsporozoite protein, CSP			X			
7	PF3D7_0206800	merozoite surface protein 2, MSP2			X			
8	PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1	X		X			
9	PF3D7_1106300	exonuclease, putative				X	X	
10	PF3D7_0423700	early transcribed membrane protein 4, ETRAMP4			X			
Top 20	PF3D7_0207000	merozoite surface protein 4, MSP4			X			
	PF3D7_0402400	Plasmodium exported protein, GEXP18				X		
	PF3D7_0501100.1	heat shock protein 40, type II, HSP40	X					
	PF3D7_0532400	Plasmodium exported protein, PHISTb						
	PF3D7_0702300	sporozoite threonine & asparagine-rich protein, STARP			X			
	PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_0936300	ring-exported protein 3, REX3						
	PF3D7_1002100	PF70 protein, PF70			X			
	PF3D7_1036400	liver stage antigen 1, LSA1			X			
	PF3D7_1129100	parasitophorous vacuolar protein 1, PV1				X		X
Top 30	PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1						
	PF3D7_0620400	merozoite surface protein 10, MSP10			X			
	PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_0930300	merozoite surface protein 1, MSP1			X			
	PF3D7_1024800	conserved Plasmodium protein			X			
	PF3D7_1100800	Maurer's cleft two transmembrane protein, MC-2TM		X	X			
	PF3D7_1133400	apical membrane antigen 1, AMA1			X		X	
	PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1	X		X			
	PF3D7_1401400	early transcribed membrane protein 14.1, ETRAMP14			X			
	PF3D7_1410400	rhoptry-associated protein 1, RAP1						

[1]Cellular Component: exported to host cell (HC); Maurer's cleft (MC); membrane/cell surface (CS); intracellular/cytoplasm/nucleus (I); apicoplast (A); symbiont-containing vacuole (SCV).

CONCLUSIONS

Malaria control, elimination (cessation of local transmission within a defined geographical area), and eradication (global disappearance of one or more species of the malaria parasite) is once again on the agenda of the international health community (31, 250). Encouragingly, the malaria burden in much of sub-Saharan Africa has declined with the scaling up of prevention, diagnosis, and treatment, advancing the initiative toward elimination. In spite of these promising advances towards malaria elimination in many countries (251, 252), malaria transmission in some regions in Africa is static or deteriorating. Surveillance is an important component of malaria control and elimination programs, and typically has relied on reporting of cases by regional health systems, entomological estimates, or parasite rates in a population. Unfortunately, as malaria transmission declines, these methodologies become less sensitive. Over the last decade, various groups have pioneered the use of serology to measure past *P. falciparum* exposure, primarily by measuring the seroconversion rate in a population (110, 196). It has been postulated that malaria control and elimination initiatives can be strengthened by the development and application of more robust serologic assays to assess malaria exposure (118). The body of work presented here represents primary steps towards the systematic and rational development of serologic assays to obtain precise, quantitative estimates of recent *P. falciparum* exposure in individuals. We believe that the development of robust serologic assays can allow for dynamic changes in exposure to be assessed over time at fine spatial scales.

The genome of *P. falciparum* contains over 5000 genes, many of which encode antigens eliciting IgG antibody responses (9, 135, 253). Importantly, different antigens tend to elicit antibody responses with different intensities and kinetics, providing a large and diverse set of potential biomarkers of exposure (205, 229, 235). Until recently, evaluation of antibody responses had been restricted to a small number of antigens, chosen for their potential as vaccine candidates rather than their ability to provide information about exposure. The recent development of protein microarray technology, which allows the evaluation of responses to hundreds or thousands of antigens simultaneously, now enables rapid evaluation of responses beyond this handful of candidates (128). While the majority of studies using this technology have focused on identification of correlates of immune protection, we recognized the potential to identify improved markers of exposure. We hypothesized that by analyzing microarray data in relation to data on recent exposure of individuals, we would identify highly informative markers of malaria exposure.

In Chapter 1, we began by reanalyzing existing data from a detailed longitudinal study in Mali, where sera from 194 individuals collected before and after the malaria transmission season was probed on a microarray containing 2,320 *P. falciparum* proteins (135). We identified putative markers of exposure via three criteria: consistent increases in antibody intensity with age, evidence of transient boosting during the transmission season that was stable across a range of ages, and ability to predict time since last infection, assuming an exponential decay of antibodies. The third criterion

was motivated by studies of other pathogens in which kinetics of antibodies have been used successfully to identify time since infection and, thereby, incidence (239–242).

Based on these findings, antigens inducing responses correlated with these three exposure metrics of interest, along with additional antigens identified from studies performed in different epidemiologic settings, were printed on an array containing 856 *P. falciparum* antigens and numerous controls for validation and normalization of responses. In Chapter 2, we probed these arrays with plasma from 186 children, aged 3-7 years, from cohort studies in two districts of Uganda: Kanungu, where exposure is moderate and seasonal, and Tororo, where exposure is perennial and intense (206, 208, 254). Importantly, participants who were more recently exposed had higher overall responses to *P. falciparum* antigens, and antibody responses were more reflective of an individual's recent malaria exposure status than which site they were located in, indicating that global responses to antigens present on our array reflected individual-level exposure. Here, putative markers of exposure were identified by their ability to predict either the number of malaria episodes each participant had within the last year and or time since last infection.

Our results on the performance of these putative serologic markers of exposure demonstrate that our selection procedure successfully identified antibody responses informative of malaria exposure, only a handful of which were required to maximize information regarding exposure. Indeed, responses to a single *P. falciparum* antigen were able to accurately predict whether or not an individual had been infected recently. These results are promising for our ultimate goal of developing practical, field-friendly assays by measuring responses to only a few antigens. The most informative responses closely followed the expected trend of increased reactivity in people who were more recently exposed, compared with the more noisy relationship in overall responses. Responses to six *P. falciparum* antigens produced predictions of incidence for each subject. Using these predictions, we were able to accurately (within 10% error) estimate the mean incidence for each site using a single serum sample from each subject, approximating small cross-sectional surveys. Furthermore, individual estimates of incidence derived from serology were able to reproduce the spatial heterogeneity of exposure observed in cohort data. Of note, a number of responses used in the past to evaluate exposure, such as apical membrane antigen 1 (AMA1), merozoite surface protein 1 (MSP1), and circumsporozoite protein (CSP) were identified as informative in our analyses, but only CSP was identified as amongst the top 10.

Future studies should build on our success with the above approach to identify responses informative in individuals across a wide range of ages and *P. falciparum* exposure intensities. Importantly, the most informative responses may not be the same in all exposure settings (110, 229, 235). For example, responses with relatively short half-lives may be well-suited for differentiating amongst individuals in a region of high exposure, while these responses may provide less information in an area of low exposure, where most individuals may have been infected too long ago for these responses to be detectable. Another important factor influencing the intensity and kinetics of antibody response is age. Age categories for inclusion should be motivated by practical considerations for ultimate scenarios of use, e.g. malaria indicator surveys (1-5 years), school surveys (6-15 years), and targeted evaluation of adults in high-risk professions. We anticipate that some but not all responses may be informative across

these age categories within a given exposure setting. Additional epidemiologic factors which may affect antibody responses are recent changes in exposure, which will allow distinction between recent and more historic exposure, and infection with other *Plasmodium* species (primarily *P. vivax)*, which may induce some cross-reactive antibodies. When developing serologic assays, it is important to take all of these factors into account and to include samples and data from a large number of longitudinal studies encompassing a range of epidemiologic settings.

Prospective studies will need to identify a limited number of serologic markers most predictive of *P. falciparum* exposure. Serum samples should be obtained from well-characterized cohort studies, so that each serum sample can be linked to detailed data on that individual's recent *P. falciparum* exposure history. These cohort studies should include subjects from infants through adults and cover a broad range of exposure intensity, from sites in elimination settings to areas around the world with the highest transmission. Numerous sites where *P. vivax* is co-endemic and sites where recent changes in exposure due to interventions have been well documented should also be included. Protein microarray technology should be utilized to perform high throughput screening of *de novo* antigenic targets useful for assessing exposure in these various populations, and not limiting the evaluation to previously studied antigens that which have been selected based on their potential for inclusion in a vaccine. Combined, these samples and data will allow for the development and validation of serologic assays for malaria exposure applicable to the broad range of epidemiologic settings where *P. falciparum* is endemic.

In addition to evaluating subjects from diverse epidemiologic settings, the reactivity and kinetics of responses to a wide range of antigens should be evaluated over time within the same subject. The presence or absence of parasites in the blood at one point in time, as given by the parasite rate, provides just one piece of information about recent malaria exposure. A richer picture of exposure can be painted by looking at a sequence of blood slides or monitoring parasite clones over time (i.e., the force of infection). Since antibody titers (intensities) wax and wane at different rates and each person's recent history of exposure is recorded within those titers, this history can be inferred from serology. By choosing antigens with titers that wax and wane at different rates, a set of titer levels can be used to estimate the infection status of a person of some known age over the past week, the past month, the past year, or over a lifetime. As with any biologic phenomenon, there will be variation in antibody kinetics within and between individuals. The detailed characterization of kinetics will allow for assessment of such variation, and methods used to choose combinations of responses should account for the trade-off between bias and variance. The measurement of multiple antibody responses within each individual and the ultimate estimation of exposure in a population from multiple individuals will mitigate the effects of this variation (240).

Once antigens for final assays are selected, appropriate assays to obtain precise quantitative measurements of antibody intensity will need to be developed and optimized. Investigations into various platforms with which to assess responses to the selected antigens will need to be conducted to compare reproducibility, precision, and accuracy. The benefits and disadvantages of laboratory versus field-based assays will need to be assessed, and antigen concentrations and serum dilutions will need to be titrated so that the greatest dynamic range and signal-to-noise ratios are obtained for

each of the assay platforms being compared. For analysis of serum samples within the environment of a central laboratory, ELISAs, bead-based arrays, or microarray technologies could be employed. ELISAs offer a number of advantages for these final assays: they are inexpensive and easy to standardize and perform in resource poor settings, where we envision these final tools will primarily be used. In addition, ELISAs allow for the flexibility to use different serum concentrations for evaluating different responses, e.g. higher dilutions may be needed for more immunogenic antigens. However, the array platforms may have several advantages over ELISAs. For example, multiple antigens can be quantitatively assayed simultaneously and independently using a small volume of serum. We and others have found that the reproducibility, noise level, and dynamic range of these platforms are as good if not better than ELISA (238). If it is determined that the analysis of multiple antigens dramatically improves precision, the potential savings in both cost and time that could be accrued by use of array-based assays in comparison to ELISAs would provide a strong incentive for the routine use of this technology in clinical laboratories (255). As an alternative, point-of-care assays that are utilized right in the field (e.g., lateral flow immunoassays) would eliminate the logistical challenges associated with transporting samples back to a central lab. Furthermore, field-based assays have the added benefit of the immediate availability of results and would enable malaria elimination programs to immediately act on field survey results, for instance in administering targeted mass drug administration to an area with evidence of recent exposure. To facilitate dissemination of final assays, simple software tools will be developed to analyze raw data, perform quality control (e.g. consistency between replicates), and transform titers into estimates of individual and population level exposure including appropriate confidence intervals around these estimates. Such software will be essential in making these assays accessible.

We believe that standardized, straightforward, and accurate serologic assays that include automated interpretation of results will provide more accurate estimates of a community's *P. falciparum* exposure, be able to more accurately detect changes in malaria exposure over time, and be better at identifying spatial hotspots of exposure than the parasite rate, as measured by both microscopy and PCR. Sero-surveillance tools should be able to provide coarse estimates of malaria exposure levels across wide geographic regions, precisely identify local hotspots of transmission on a small spatial scale, monitor changes in exposure over time, evaluate the impact of public health interventions, certify malaria elimination, and monitor for malaria re-emergence.

At large spatial scales, malaria transmission occurs in regions where the climate, environment, or ecology is well suited to the local vector and parasite populations (178). As geographic distribution of malaria is not homogeneously distributed, most countries will contain regions with areas of higher and lower transmission intensity. Thus, a tailored approach to assess malaria exposure is needed so that tools appropriate to each exposure setting are utilized. Local epidemiological and logistical factors will influence which intervention(s) will be implemented in each district. Different interventions are essential for effective resource utilization in areas of high, moderate, and low *P. falciparum* exposure.

In control (pre-elimination) and early elimination phases, interventions should be targeted to broad regions of high malaria exposure. Sero-surveillance tools can be used by control programs to identify areas with the highest malaria exposure. In high

transmission settings, malaria control relies on effective prevention and case management. Sero-surveillance tools capable of identifying broad regions of a country with the highest malaria exposure would allow interventions for vector control, such as indoor residual spraying or insecticide-treated net distribution, or for mass drug administration, such as intermittent preventive treatment in infants, children, or pregnant women, to be targeted to areas with the greatest need.

"Hotspots," geographically discrete households or groups of households that maintain significantly higher malaria transmission rates than surrounding areas, can be found within general geographical areas supporting malaria transmission (160, 166, 230, 243). Even before an area enters the pre-elimination phase, heterogeneity of malaria exposure within a village is present (256). As previous investigations have demonstrated that hotspots are relatively resilient to indiscriminate control efforts and sustain stable malaria exposure even when overall malaria transmission is reduced, hotspots are likely to be areas where residual malaria transmission will persist in the absence of a directed intervention (230, 257, 258). Furthermore, as hotspots of transmission are a source of infection for entire communities, directing interventions at hotspots has the potential to reduce malaria exposure across a village (259, 260). Variations in malaria exposure on a small scale have been defined by serologic assays (104, 260, 261), and it is possible that more fine-scale analyses of exposure heterogeneity may be obtained from a tuned approach.

As exposure intensity declines and malaria control programs focus their efforts towards elimination, it becomes increasingly important to be able to identify these asymptomatically infected individuals in order to interrupt transmission. Passive surveillance systems can identify, treat, and report cases of symptomatic malaria infection. However, the majority of *Plasmodium* infections—in both high and low exposure settings—are asymptomatic (262–264). These asymptomatically infected individuals represent an important reservoir of gametocytes that are infectious to mosquitos. Serology, which can provide evidence of recent or past infection, can be used to identify geographic areas at higher risk of malaria exposure.

In areas where the interruption of transmission has been accomplished, continuous monitoring is necessary to be able to maintain elimination status by promptly detecting and treating reintroduced malaria cases before an outbreak has the chance to expand (251). The development of novel sero-surveillance tools for this purpose is now a high priority. Additionally, sensitive serologic assays that can rapidly screen subjects for *Plasmodium* infection before entering malaria-free areas would prevent reintroduction of the disease. Finally, a finely tuned serologic assay could provide evidence needed for the certification of malaria elimination, the official recognition of a country's malaria-free status by the World Health Organization (256).

This dissertation has demonstrated that antibody responses to *P. falciparum* antigens selected for associations with previous malaria exposure have diverse kinetic profiles. These differences in the rates of antibody acquisition and decay may prove to be useful for the creation of robust serologic tools that have functionality across various malaria exposure settings. Development of these serologic tools will require extensive experimentation and validation across various malaria exposure settings. However, the methodologies outlined here will allow for high-throughput selection of serologic biomarkers of exposure in areas with diverse epidemiologies. In addition to public health

applications, serologic estimates of exposure can provide a valuable research tool. To be valuable in this context, consideration would need to be taken to identify markers of exposure not directly involved in mediating immune protection or strongly influenced by blood stage immunity.

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Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria.

Gene ID	Description	Spot ID
PF3D7_0102200	ring-infected erythrocyte surface antigen (RESA)	PFA0110w.e1.s1
PF3D7_0102200	ring-infected erythrocyte surface antigen (RESA)	PFA0110w.e2.s2
PF3D7_0102500	erythrocyte binding antigen-181 (EBA181)	PFA0125c.e1.s1
PF3D7_0102500	erythrocyte binding antigen-181 (EBA181)	PFA0125c.e1.s2
PF3D7_0103400	zinc-carboxypeptidase	PFA0170c.e1.s1
PF3D7_0103400	zinc-carboxypeptidase	PFA0170c.e1.s2
PF3D7_0103800	actin-related protein (ARP1)	PFA0190c.e4.s1
PF3D7_0104600	conserved Plasmodium protein	PFA0235w.e1.s1
PF3D7_0105200	conserved Plasmodium protein	PFA0255c.e1.s1
PF3D7_0105600	conserved Plasmodium protein	PFA0275c.e2
PF3D7_0105600	conserved Plasmodium protein	PFA0275c.e3
PF3D7_0106700	small ribosomal subunit assembling AARP2 protein (AARP2)	PFA0330w.s1
PF3D7_0107100	conserved Plasmodium protein	PFA0350c
PF3D7_0107600	serine/threonine protein kinase	PFA0380w.e1.s2
PF3D7_0108000	beta3 proteasome subunit	PFA0400c.e1.s1
PF3D7_0108300	conserved Plasmodium protein	PFA0410w.s1
PF3D7_0108300	conserved Plasmodium protein	PFA0410w.s2
PF3D7_0108300	conserved Plasmodium protein	PFA0410w.s3
PF3D7_0108700	secreted ookinete protein (PSOP24)	PFA0430c.e1.s1
PF3D7_0108700	secreted ookinete protein (PSOP24)	PFA0430c.e1.s2
PF3D7_0110500	bromodomain protein	PFA0510w.e1.s2
PF3D7_0110500	bromodomain protein	PFA0510w.e1.s3
PF3D7_0110600	phosphatidylinositol-4-phosphate 5-kinase (PIP5K)	PFA0515w.e1.s1
PF3D7_0110600	phosphatidylinositol-4-phosphate 5-kinase (PIP5K)	PFA0515w.e1.s2
PF3D7_0110600	phosphatidylinositol-4-phosphate 5-kinase (PIP5K)	PFA0515w.e3
PF3D7_0110700	chromatin assembly factor 1 protein WD40 domain	PFA0520c.e1.s1
PF3D7_0111000	kinesin-8	PFA0535c.e1.s2
PF3D7_0111500	UMP-CMP kinase	PFA0555c.e1.s1
PF3D7_0111500	UMP-CMP kinase	PFA0555c.e4.s1
PF3D7_0111500	UMP-CMP kinase	PFA0555c.e5.s1
PF3D7_0114000	RESA-like protein with DnaJ domain (GEXP06)	PFA0675w.e2.s2
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3)	PFA0725w.e1.s1
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3)	PFA0725w.e2.s1
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3)	PFA0725w.e3.s1
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3)	PFA0725w.e3.s2
PF3D7_0115100	Plasmodium exported protein (PHISTa)	PFA0735w.e2.s1
PF3D7_0202000	knob-associated histidine-rich protein (KAHRP)	PFB0100c.e2.s1
PF3D7_0202400	conserved Plasmodium protein	PFB0115w.e1.s1
PF3D7_0202400	conserved Plasmodium protein	PFB0115w.e1.s2
PF3D7_0203100; PF3D7_0203200	protein kinase; conserved Plasmodium protein	PFB0150c.e1.s1
PF3D7_0203100; PF3D7_0203200	protein kinase; conserved Plasmodium protein	PFB0150c.e2.s1
PF3D7_0203100; PF3D7_0203200	protein kinase; conserved Plasmodium protein	PFB0150c.e2.s2
PF3D7_0203100; PF3D7_0203200	protein kinase; conserved Plasmodium protein	PFB0150c.e2.s3
PF3D7_0204200	conserved Plasmodium protein	PFB0194w.e1.s1
PF3D7_0204200	conserved Plasmodium protein	PFB0194w.e2.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0205900	proteasome 26S regulatory subunit	PFB0260w.e2
PF3D7_0206000	DNA repair endonuclease	PFB0265c.s2
PF3D7_0206500	conserved Plasmodium protein	PFB0285c.e1.s2
PF3D7_0206800	merozoite surface protein 2 (MSP2)	PFB0300c
PF3D7_0206900.1; PF3D7_0206900.2	merozoite surface protein 5 (MSP5)	PFB0305c.e1
PF3D7_0207000	merozoite surface protein 4 (MSP4)	PFB0310c.e1
PF3D7_0207100	conserved Plasmodium protein	PFB0315w.s1
PF3D7_0207500	serine repeat antigen 6 (SERA6)	PFB0335c.e1.s1
PF3D7_0207500	serine repeat antigen 6 (SERA6)	PFB0335c.e3.s1
PF3D7_0207600	serine repeat antigen 5 (SERA5)	PFB0340c.e2.s1
PF3D7_0207600	serine repeat antigen 5 (SERA5)	PFB0340c.e4.s1
PF3D7_0208900	6-cysteine protein (P230p)	PFB0400w.e1.s1
PF3D7_0208900	6-cysteine protein (P230p)	PFB0400w.e1.s3
PF3D7_0209000	6-cysteine protein (P230)	PFB0405w.s1
PF3D7_0209000	6-cysteine protein (P230)	PFB0405w.s3
PF3D7_0209000	6-cysteine protein (P230)	PFB0405w.s4
PF3D7_0209800	ATP-dependent RNA helicase UAP56 (UAP56)	PFB0445c.e2
PF3D7_0210300	monocarboxylate transporter	PFB0465c.e1
PF3D7_0210300	monocarboxylate transporter	PFB0465c.e2
PF3D7_0212100	conserved Plasmodium protein	PFB0540w.e1.s2
PF3D7_0212300	peptide chain release factor subunit 1	PFB0550w.e3.s1
PF3D7_0213100	heat shock protein 40	PFB0595w.e3.s1
PF3D7_0213700	conserved protein	PFB0620w.e1.s1
PF3D7_0214000	T-complex protein 1	PFB0635w.e1.s1
PF3D7_0214100	protein transport protein sec31 (SEC31)	PFB0640c.e1.s1
PF3D7_0214100	protein transport protein sec31 (SEC31)	PFB0640c.e1.s2
PF3D7_0214200	mitochondrial ribosomal protein L13 precursor	PFB0645c.e2
PF3D7_0214900	rhoptry neck protein 6 (RON6)	PFB0680w.e2.s1
PF3D7_0215500	conserved Plasmodium protein	PFB0705w.e1.s1
PF3D7_0216000	DEAD/DEAH box helicase	PFB0730w.e1.s1
PF3D7_0217900	conserved Plasmodium protein	PFB0835c.e1.s1
PF3D7_0218000	replication factor C, subunit 2	PFB0840w.e1.s1
PF3D7_0218200; PF3D7_0218300	conserved Plasmodium protein; apicoplast RNA methyltransferase precursor	PFB0855c.e2
PF3D7_0218200; PF3D7_0218300	conserved Plasmodium protein; apicoplast RNA methyltransferase precursor	PFB0855c.e5
PF3D7_0219600	replication factor C subunit 1	PFB0895c.e1.s1
PF3D7_0220000	liver stage antigen 3 (LSA3)	PFB0915w.e2.s1
PF3D7_0220000	liver stage antigen 3 (LSA3)	PFB0915w.e2.s2
PF3D7_0220100	DnaJ protein	PFB0920w.e1.s1
PF3D7_0301700	Plasmodium exported protein	PFC0085c.e1.s1
PF3D7_0301700	Plasmodium exported protein	PFC0085c.e2.s1
PF3D7_0302200	cytoadherence linked asexual protein 3.2 (CLAG3.2)	PFC0110w.e1
PF3D7_0302200	cytoadherence linked asexual protein 3.2 (CLAG3.2)	PFC0110w.e2
PF3D7_0302200	cytoadherence linked asexual protein 3.2 (CLAG3.2)	PFC0110w.e8
PF3D7_0302500	cytoadherence linked asexual protein 3.1 (CLAG3.1)	PFC0120w.e1.s1
PF3D7_0302500	cytoadherence linked asexual protein 3.1 (CLAG3.1)	PFC0120w.e2.s1
PF3D7_0302500	cytoadherence linked asexual protein 3.1 (CLAG3.1)	PFC0120w.e6.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0302500	cytoadherence linked asexual protein 3.1 (CLAG3.1)	PFC0120w.e8.s1
PF3D7_0302800	conserved Plasmodium protein	PFC0130c.e1.s1
PF3D7_0302900	exportin 1	PFC0135c.e1.s1
PF3D7_0303200	HAD superfamily protein	PFC0150w.e2.s1
PF3D7_0303200	HAD superfamily protein	PFC0150w.e2.s2
PF3D7_0303700	dihydrolipoamide acyltransferase	PFC0170c.e1.s1
PF3D7_0303900	conserved Plasmodium protein	PFC0176c.e2.s1
PF3D7_0304000	inner membrane complex protein 1a (IMC1a)	PFC0180c.e4.s1
PF3D7_0304200	EH (Eps15 homology) protein (PAST1)	PFC0190c.e1.s1
PF3D7_0304600	circumsporozoite (CS) protein (CSP)	PFC0210c
PF3D7_0304800	conserved Plasmodium membrane protein	PFC0220w.e1.s2
PF3D7_0305100	conserved Plasmodium protein	PFC0230c.e1.s2
PF3D7_0305100	conserved Plasmodium protein	PFC0230c.e1.s3
PF3D7_0305100	conserved Plasmodium protein	PFC0230c.e1.s4
PF3D7_0305500	conserved Plasmodium protein	PFC0245c.e1.s1
PF3D7_0305500	conserved Plasmodium protein	PFC0245c.e1.s3
PF3D7_0306400	FAD-dependent glycerol-3-phosphate dehydrogenase	PFC0275w.e1.s1
PF3D7_0306500	conserved Plasmodium protein	PFC0280c.e1.s1
PF3D7_0306800	T-complex protein beta subunit	PFC0285c.e1.s1
PF3D7_0307700	conserved Plasmodium protein	PFC0325c.e3.s1
PF3D7_0307700	conserved Plasmodium protein	PFC0325c.e3.s2
PF3D7_0307800	conserved Plasmodium protein	PFC0330w
PF3D7_0308300	conserved Plasmodium protein	PFC0355c.e1.s1
PF3D7_0310200	phd finger protein	PFC0425w.e1.s1
PF3D7_0310200	phd finger protein	PFC0425w.e1.s3
PF3D7_0310200	phd finger protein	PFC0425w.e1.s4
PF3D7_0310300	phosphoglycerate mutase	PFC0430w.e1.s1
PF3D7_0310300	phosphoglycerate mutase	PFC0430w.e1.s2
PF3D7_0310500	DEAD box helicase	PFC0440c.e1.s1
PF3D7_0310500	DEAD box helicase	PFC0440c.e1.s2
PF3D7_0310500	DEAD box helicase	PFC0440c.e1.s3
PF3D7_0310800	conserved Plasmodium protein	PFC0450w
PF3D7_0311800	conserved protein	PFC0500w.e3
PF3D7_0314200; PF3D7_0314300	conserved Plasmodium protein; DER1-like protein (Der1-2)	PFC0590c.e1
PF3D7_0314200; PF3D7_0314300	conserved Plasmodium protein; DER1-like protein (Der1-2)	PFC0590c.e2.s1
PF3D7_0314200; PF3D7_0314300	conserved Plasmodium protein; DER1-like protein (Der1-2)	PFC0590c.e2.s2
PF3D7_0314400	serine/threonine protein phosphatase	PFC0595c.e1.s1
PF3D7_0315100	translation initiation factor 4E (eIF4E)	PFC0635c.e1.s1
PF3D7_0316000	conserved Plasmodium protein	PFC0700c
PF3D7_0316300.1; PF3D7_0316300.2	inorganic pyrophosphatase	PFC0710w.e1
PF3D7_0316300.1; PF3D7_0316300.2	inorganic pyrophosphatase	PFC0710w.e3
PF3D7_0316500	conserved Plasmodium protein	PFC0720w.e1.s1
PF3D7_0317000	proteasome component C8	PFC0745c.e1.s1
PF3D7_0317300	conserved Plasmodium protein	PFC0760c.s3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0317600	40S ribosomal protein S11	PFC0775w.e2.s1
PF3D7_0317700	CPSF (cleavage and polyadenylation specific factor), subunit A	PFC0780w.e1.s1
PF3D7_0317700	CPSF (cleavage and polyadenylation specific factor), subunit A	PFC0780w.e1.s2
PF3D7_0317700	CPSF (cleavage and polyadenylation specific factor), subunit A	PFC0780w.e9.s1
PF3D7_0318100	stomatin-like protein	PFC0800w.e1.s1
PF3D7_0318200	DNA-directed RNA polymerase II	PFC0805w.e1.s1
PF3D7_0318200	DNA-directed RNA polymerase II	PFC0805w.e1.s2
PF3D7_0318200	DNA-directed RNA polymerase II	PFC0805w.e1.s3
PF3D7_0318300	conserved Plasmodium protein	PFC0810c.e2.s1
PF3D7_0318300	conserved Plasmodium protein	PFC0810c.e3.s1
PF3D7_0319700	ABC transporter	PFC0875w.e1.s1
PF3D7_0319700	ABC transporter	PFC0875w.e1.s2
PF3D7_0320400	oocyst capsule protein (Cap380)	PFC0905c.e1.s3
PF3D7_0320400	oocyst capsule protein (Cap380)	PFC0905c.e1.s4
PF3D7_0320800	ATP-dependent RNA helicase (DOZI)	PFC0915w.e1.s1
PF3D7_0320800	ATP-dependent RNA helicase (DOZI)	PFC0915w.e2.s1
PF3D7_0321600	ATP-dependent RNA helicase	PFC0955w.e1
PF3D7_0322000	peptidyl-prolyl cis-trans isomerase (CYP19A)	PFC0975c.e1.s1
PF3D7_0323600	conserved Plasmodium protein	PFC1055w.e1
PF3D7_0323800	conserved Plasmodium protein	PFC1065w.e2.s1
PF3D7_0402000	Plasmodium exported protein (PHISTa)	PFD0090c.e1.s1
PF3D7_0402100	Plasmodium exported protein (PHISTb)	PFD0095c.e2.s1
PF3D7_0402200	surface-associated interspersed protein 4.1 (SURF4.1)	PFD0105c.e1.s1
PF3D7_0404600	conserved Plasmodium membrane protein	PFD0225w.s1
PF3D7_0404600	conserved Plasmodium membrane protein	PFD0225w.s3
PF3D7_0404600	conserved Plasmodium membrane protein	PFD0225w.s5
PF3D7_0404800	conserved Plasmodium protein	PFD0235c.e1
PF3D7_0405400	pre-mRNA-processing-splicing factor 8 (PRPF8)	PFD0265w.e2.s1
PF3D7_0405400	pre-mRNA-processing-splicing factor 8 (PRPF8)	PFD0265w.e2.s2
PF3D7_0405900	apical sushi protein (ASP)	PFD0295c.e3.s1
PF3D7_0405900	apical sushi protein (ASP)	PFD0295c.e4.s1
PF3D7_0406100	vacuolar ATP synthase subunit b	PFD0305c.e2.s1
PF3D7_0406200	sexual stage-specific protein precursor (Pfs16)	PFD0310w
PF3D7_0407700	conserved Plasmodium protein	PFD0380c.e2.s1
PF3D7_0407700	conserved Plasmodium protein	PFD0380c.e2.s2
PF3D7_0407800	conserved Plasmodium protein	PFD0385w.e1.s2
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	PFD0430c.e1.s1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	PFD0430c.e2.s1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	PFD0430c.e3.s1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	PFD0430c.e7.s1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	PFD0430c.e8.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0408900.1; PF3D7_0408900.2	peptidase, M22 family	PFD0440w.e1.s1
PF3D7_0408900.1; PF3D7_0408900.2	peptidase, M22 family	PFD0440w.e2.s1
PF3D7_0408900.1; PF3D7_0408900.2	peptidase, M22 family	PFD0440w.e3.s1
PF3D7_0409000	conserved Plasmodium protein	PFD0445c.s1
PF3D7_0409000	conserved Plasmodium protein	PFD0445c.s2
PF3D7_0409000	conserved Plasmodium protein	PFD0445c.s3
PF3D7_0409100	U4/U6 small nuclear ribonucleoprotein PRP31 (PRPF31)	PFD0450c.e2
PF3D7_0409800	zinc finger protein	PFD0485w
PF3D7_0413600	26S proteasome AAA-ATPase subunit RPT3	PFD0665c.e2
PF3D7_0414000	chromosome associated protein	PFD0685c.e2.s1
PF3D7_0414000	chromosome associated protein	PFD0685c.e7.s1
PF3D7_0416800	small GTP-binding protein sar1 (SAR1)	PFD0810w.e3.s1
PF3D7_0419600	ran binding protein 1	PFD0950w.e2.s1
PF3D7_0420000	zinc finger protein	PFD0970c.s2
PF3D7_0420000	zinc finger protein	PFD0970c.s3
PF3D7_0420000	zinc finger protein	PFD0970c.s4
PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1 (VAR)	PFD0995c.e1.s2
PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1 (VAR)	PFD0995c.e2.s1
PF3D7_0422400	40S ribosomal protein S19	PFD1055w.e2.s1
PF3D7_0422500	pre-mRNA-splicing helicase BRR2 (BRR2)	PFD1060w.e1.s1
PF3D7_0422500	pre-mRNA-splicing helicase BRR2 (BRR2)	PFD1060w.e1.s2
PF3D7_0422500	pre-mRNA-splicing helicase BRR2 (BRR2)	PFD1060w.e1.s3
PF3D7_0422700	eukaryotic initiation factor	PFD1070w.e1.s1
PF3D7_0424600	Plasmodium exported protein (PHISTb)	PFD1170c.e1.s1
PF3D7_0424600	Plasmodium exported protein (PHISTb)	PFD1170c.e2.s1
PF3D7_0500800	mature parasite-infected erythrocyte surface antigen,erythrocyte membrane protein 2 (MESA)	PFE0040c.e1.s1
PF3D7_0500800	mature parasite-infected erythrocyte surface antigen,erythrocyte membrane protein 2 (MESA)	PFE0040c.e2.s1
PF3D7_0501000	Plasmodium exported protein	PFE0050w.e1.s1
PF3D7_0501100.1; PF3D7_0501100.2	heat shock protein 40, type II (HSP40)	PFE0055c.e3.s1
PF3D7_0501100.1; PF3D7_0501100.2	heat shock protein 40, type II (HSP40)	PFE0055c.e4.s1
PF3D7_0501200	parasite-infected erythrocyte surface protein (PIESP2)	PFE0060w.e2
PF3D7_0501500	rhoptry-associated protein 3 (RAP3)	PFE0075c.e1.s1
PF3D7_0501600	rhoptry-associated protein 2 (RAP2)	PFE0080c.e1.s1
PF3D7_0501800	chromosome assembly factor 1 (CAF1)	PFE0090w.e1.s1
PF3D7_0501800	chromosome assembly factor 1 (CAF1)	PFE0090w.e1.s2
PF3D7_0503400	actin-depolymerizing factor 1 (ADF1)	PFE0165w.e1.s1
PF3D7_0503600	myosin B (MyoB)	PFE0175c.e1.s1
PF3D7_0503600	myosin B (MyoB)	PFE0175c.e5.s1
PF3D7_0504600	3-methyl-2-oxobutanoate dehydrogenase (lipoamide)	PFE0225w
PF3D7_0505200	actin-like protein (ALP2b)	PFE0255w.e1.s1
PF3D7_0505400	conserved Plasmodium protein	PFE0265c
PF3D7_0505500	DNA repair protein	PFE0270c.e1.s1
PF3D7_0506800	transcription factor 25 (TCF25)	PFE0335w.e1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0507300	conserved Plasmodium protein	PFE0360c
PF3D7_0507700	nuclear protein localization protein 4 (NPL4)	PFE0380c.e1.s1
PF3D7_0507800	conserved Plasmodium protein	PFE0385w.s2
PF3D7_0508500	guanidine nucleotide exchange factor (RCC1)	PFE0420c.s1
PF3D7_0508500	guanidine nucleotide exchange factor (RCC1)	PFE0420c.s3
PF3D7_0509000	SNAP protein (soluble N-ethylmaleimide-sensitive factor Attachment Protein)	PFE0445c.e5.s1
PF3D7_0509400	RNA polymerase I (RNAPI)	PFE0465c.e1.s1
PF3D7_0509400	RNA polymerase I (RNAPI)	PFE0465c.e1.s3
PF3D7_0509700	conserved Plasmodium protein	PFE0480c.e4.s1
PF3D7_0511200	stearoyl-CoA desaturase (SCD)	PFE0555w.e1
PF3D7_0511200	stearoyl-CoA desaturase (SCD)	PFE0555w.e2
PF3D7_0511400	conserved Plasmodium protein	PFE0565w
PF3D7_0511500	RNA pseudouridylate synthase	PFE0570w.s1
PF3D7_0511500	RNA pseudouridylate synthase	PFE0570w.s11
PF3D7_0511500	RNA pseudouridylate synthase	PFE0570w.s3
PF3D7_0511500	RNA pseudouridylate synthase	PFE0570w.s4
PF3D7_0511500	RNA pseudouridylate synthase	PFE0570w.s9
PF3D7_0511800	myo-inositol 1-phosphate synthase	PFE0585c.e2
PF3D7_0512200	glutathione synthetase (GS)	PFE0605c
PF3D7_0512800	conserved Plasmodium protein	PFE0635c.e1.s2
PF3D7_0513000	conserved Plasmodium protein	PFE0645w.e1.s1
PF3D7_0513200	conserved Plasmodium protein	PFE0655w.e1.s1
PF3D7_0513200	conserved Plasmodium protein	PFE0655w.e2.s2
PF3D7_0513200	conserved Plasmodium protein	PFE0655w.e2.s3
PF3D7_0513300	purine nucleoside phosphorylase (PNP)	PFE0660c.e1.s1
PF3D7_0513600	deoxyribodipyrimidine photo-lyase	PFE0675c.e1.s1
PF3D7_0513700	secreted ookinete protein (PSOP12)	PFE0680w.e3.s1
PF3D7_0514700	conserved Plasmodium protein	PFE0735w.e1.s2
PF3D7_0515000	RNA binding protein	PFE0750c.e1.s1
PF3D7_0515300	phosphatidylinositol 3-kinase (PI3K)	PFE0765w.s1
PF3D7_0515300	phosphatidylinositol 3-kinase (PI3K)	PFE0765w.s2
PF3D7_0515300	phosphatidylinositol 3-kinase (PI3K)	PFE0765w.s3
PF3D7_0515600	conserved Plasmodium protein	PFE0780w.e6.s1
PF3D7_0515600	conserved Plasmodium protein	PFE0780w.e8.s1
PF3D7_0516100	cation-transporting ATPase 1 (ATPase1)	PFE0805w.e1.s1
PF3D7_0516100	cation-transporting ATPase 1 (ATPase1)	PFE0805w.e1.s2
PF3D7_0516100	cation-transporting ATPase 1 (ATPase1)	PFE0805w.e1.s3
PF3D7_0516100	cation-transporting ATPase 1 (ATPase1)	PFE0805w.e2.s1
PF3D7_0516600	translation initiation factor IF-2, sporozoite surface antigen MB2 (MB2)	PFE0830c.s1
PF3D7_0516600	translation initiation factor IF-2, sporozoite surface antigen MB2 (MB2)	PFE0830c.s2
PF3D7_0516900	60S ribosomal protein L8	PFE0845c.e2
PF3D7_0517300	pre-mRNA-splicing factor (SR1)	PFE0865c.e1
PF3D7_0517400	FACT complex subunit SPT16 (FACT-L)	PFE0870w.e1.s1
PF3D7_0517400	FACT complex subunit SPT16 (FACT-L)	PFE0870w.e1.s2
PF3D7_0517700	eukaryotic translation initiation factor	PFE0885w
PF3D7_0518300	proteasome subunit beta type 1	PFE0915c.e4.s1
PF3D7_0518700	mRNA-binding protein PUF1 (PUF1)	PFE0935c.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0518700	mRNA-binding protein PUF1 (PUF1)	PFE0935c.e3.s1
PF3D7_0519200	vacuolar ATP synthetase	PFE0965c.e2.s1
PF3D7_0519400	40S ribosomal protein S24	PFE0975c.e1.s1
PF3D7_0519500	carbon catabolite repressor protein 4 (CCR4)	PFE0980c.e1.s3
PF3D7_0519800	conserved protein	PFE0995c.e2.s1
PF3D7_0520000	40S ribosomal protein S9	PFE1005w.e3.s1
PF3D7_0520100	protein phosphatase	PFE1010w.e1.s1
PF3D7_0520400	conserved Plasmodium protein	PFE1025c.e1.s1
PF3D7_0521700	DEAD/DEAH box ATP-dependent RNA helicase	PFE1085w.e1.s1
PF3D7_0521900	conserved Plasmodium protein	PFE1095w.e2.s1
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s1
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s2
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s3
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s5
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s6
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e3.s7
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e4.s1
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e4.s2
PF3D7_0522400	conserved Plasmodium protein	PFE1120w.e4.s3
PF3D7_0522800	G10 protein	PFE1140c.e1.s1
PF3D7_0522900	zinc finger protein	PFE1145w.e1.s1
PF3D7_0523000	multidrug resistance protein (MDR1)	PFE1150w.e1.s1
PF3D7_0523000	multidrug resistance protein (MDR1)	PFE1150w.e1.s2
PF3D7_0524000	karyopherin beta (KASbeta)	PFE1195w
PF3D7_0524400	cytosolic preribosomal GTP-binding protein	PFE1215c.e1.s1
PF3D7_0525100	acyl-CoA synthetase (ACS10)	PFE1250w.e1.s1
PF3D7_0525800	membrane skeletal protein IMC1-related	PFE1285w.e1.s1
PF3D7_0526500	conserved Plasmodium protein	PFE1320w.s1
PF3D7_0526500	conserved Plasmodium protein	PFE1320w.s2
PF3D7_0527200	ubiquitin carboxyl-terminal hydrolase	PFE1355c.e2.s1
PF3D7_0528200	eukaryotic translation initiation factor 3, subunit 6	PFE1405c.e2.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e1.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e2.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e3.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e5.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e7.s1
PF3D7_0529400.1; PF3D7_0529400.2	conserved Plasmodium protein	PFE1465w.e9.s1
PF3D7_0532100	early transcribed membrane protein 5 (ETRAMP5)	PFE1590w
PF3D7_0532300	Plasmodium exported protein (PHISTb)	PFE1600w.e1.s1
PF3D7_0532300	Plasmodium exported protein (PHISTb)	PFE1600w.e2.s1
PF3D7_0532400	Plasmodium exported protein (PHISTb)	PFE1605w.e1.s1
PF3D7_0532400	Plasmodium exported protein (PHISTb)	PFE1605w.e2.s1
PF3D7_0617200	conserved Plasmodium protein	MAL6P1.254.e3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	MAL6P1.252.e1.s1
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	MAL6P1.252.e1.s2
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	MAL6P1.252.e1.s3
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	MAL6P1.252.e2
PF3D7_0619000.1; PF3D7_0619000.2	conserved Plasmodium protein	MAL6P1.237.e12
PF3D7_0619000.1; PF3D7_0619000.2	conserved Plasmodium protein	MAL6P1.237.e6.s1
PF3D7_0619000.1; PF3D7_0619000.2	conserved Plasmodium protein	MAL6P1.237.e6.s2
PF3D7_0622800	leucine--tRNA ligase	MAL6P1.201.s1
PF3D7_0622800	leucine--tRNA ligase	MAL6P1.201.s2
PF3D7_0628200	protein kinase PK4 (PK4)	MAL6P1.146.s2
PF3D7_0628200	protein kinase PK4 (PK4)	MAL6P1.146.s3
PF3D7_0628200	protein kinase PK4 (PK4)	MAL6P1.146.s4
PF3D7_0629700	SET domain protein (SET1)	MAL6P1.131.e1.s1
PF3D7_0629700	SET domain protein (SET1)	MAL6P1.131.e1.s3
PF3D7_0629700	SET domain protein (SET1)	MAL6P1.131.e1.s4
PF3D7_0629700	SET domain protein (SET1)	MAL6P1.131.e1.s5
PF3D7_0629700	SET domain protein (SET1)	MAL6P1.131.e1.s7
PF3D7_0701900	Plasmodium exported protein	PF07.0004.e2.s1
PF3D7_0702300	sporozoite threonine and asparagine-rich protein (STARP)	PF07.0006.e2
PF3D7_0702400	conserved Plasmodium protein	PF07.0007.e1.s1
PF3D7_0703500	erythrocyte membrane-associated antigen	MAL7P1.12.e2.s1
PF3D7_0703500	erythrocyte membrane-associated antigen	MAL7P1.12.e2.s2
PF3D7_0703500	erythrocyte membrane-associated antigen	MAL7P1.12.e2.s3
PF3D7_0703700	conserved Plasmodium protein	MAL7P1.14.e1.s1
PF3D7_0704300	conserved Plasmodium membrane protein	PF07.0016.s1
PF3D7_0705000	methyltransferase	PF07.0020.e1.s1
PF3D7_0705000	methyltransferase	PF07.0020.e1.s2
PF3D7_0705000	methyltransferase	PF07.0020.e2
PF3D7_0707800	RAP protein	MAL7P1.23.e1.s1
PF3D7_0707800	RAP protein	MAL7P1.23.e1.s2
PF3D7_0708000	cytoskeleton associated protein	MAL7P1.25.e1.s1
PF3D7_0708000	cytoskeleton associated protein	MAL7P1.25.e2.s1
PF3D7_0708300	O-sialoglycoprotein endopeptidase	MAL7P1.26.e2
PF3D7_0708400	heat shock protein 90 (HSP90)	PF07.0029.e1
PF3D7_0708400	heat shock protein 90 (HSP90)	PF07.0029.e2
PF3D7_0709000	chloroquine resistance transporter (CRT)	MAL7P1.27.e3.s1
PF3D7_0709100	Cg1 protein	PF07.0035.e1.s1
PF3D7_0709100	Cg1 protein	PF07.0035.e1.s2
PF3D7_0709700	lysophospholipase	PF07.0040.e1.s1
PF3D7_0709900	conserved Plasmodium membrane protein	MAL7P1.29.e1.s1
PF3D7_0709900	conserved Plasmodium membrane protein	MAL7P1.29.e1.s2
PF3D7_0709900	conserved Plasmodium membrane protein	MAL7P1.29.e2
PF3D7_0709900	conserved Plasmodium membrane protein	MAL7P1.29.e3
PF3D7_0710000	conserved Plasmodium protein	MAL7P1.30.e1.s1
PF3D7_0710000	conserved Plasmodium protein	MAL7P1.30.e2.s1
PF3D7_0710000	conserved Plasmodium protein	MAL7P1.30.e5.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0710000	conserved Plasmodium protein	MAL7P1.30.e6.s1
PF3D7_0710000	conserved Plasmodium protein	MAL7P1.30.e7.s2
PF3D7_0710400	nucleotide excision repair protein	MAL7P1.32
PF3D7_0711000	AAA family ATPase, CDC48 subfamily (Cdc48)	PF07.0047.e1.s1
PF3D7_0711000	AAA family ATPase, CDC48 subfamily (Cdc48)	PF07.0047.e1.s2
PF3D7_0711500	regulator of chromosome condensation	MAL7P1.38.e1.s1
PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF07.0048.e1.s2
PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF07.0048.e2.s1
PF3D7_0713900	conserved Plasmodium protein	PF07.0053.e1.s1
PF3D7_0713900	conserved Plasmodium protein	PF07.0053.e1.s3
PF3D7_0713900	conserved Plasmodium protein	PF07.0053.e1.s4
PF3D7_0715200	conserved Plasmodium protein	PF07.0061.e1.s1
PF3D7_0715200	conserved Plasmodium protein	PF07.0061.e1.s3
PF3D7_0716300	conserved Plasmodium protein	MAL7P1.77.e1.s1
PF3D7_0716800	eukaryotic translation initiation factor 3 37.28 kDa subunit	MAL7P1.81.e3.s1
PF3D7_0717500	calcium-dependent protein kinase 4 (CDPK4)	PF07.0072.e1.s1
PF3D7_0717500	calcium-dependent protein kinase 4 (CDPK4)	PF07.0072.e2.s1
PF3D7_0717700	serine--tRNA ligase	PF07.0073.e1.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e11.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e3.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e4.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e6.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e8.s1
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e9.s3
PF3D7_0718000	dynein heavy chain	MAL7P1.89.e9.s4
PF3D7_0718300	cysteine repeat modular protein 2 (CRMP2)	MAL7P1.92.e5.s1
PF3D7_0718300	cysteine repeat modular protein 2 (CRMP2)	MAL7P1.92.e6.s1
PF3D7_0718300	cysteine repeat modular protein 2 (CRMP2)	MAL7P1.92.e7.s2
PF3D7_0719200	NIMA related kinase 4 (NEK4)	MAL7P1.100.e1.s1
PF3D7_0719200	NIMA related kinase 4 (NEK4)	MAL7P1.100.e9.s1
PF3D7_0719400	conserved Plasmodium protein	MAL7P1.102.s1
PF3D7_0719400	conserved Plasmodium protein	MAL7P1.102.s2
PF3D7_0720400	ferrodoxin reductase-like protein	PF07.0085.e1.s1
PF3D7_0721100	conserved Plasmodium protein	PF07.0087
PF3D7_0721600	40S ribosomal protein S5	PF07.0088.e2.s1
PF3D7_0721700	secreted ookinete protein (PSOP1)	PF07.0089
PF3D7_0722400	GTP binding protein	MAL7P1.122.e3.s1
PF3D7_0722600	nucleolar rRNA processing protein	PF07.0092.e1.s1
PF3D7_0723400	conserved Plasmodium protein	PF07.0097.e1.s1
PF3D7_0723400	conserved Plasmodium protein	PF07.0097.e1.s2
PF3D7_0723900	conserved Plasmodium protein	MAL7P1.126.e1.s2
PF3D7_0724700	conserved Plasmodium protein	MAL7P1.134.e1.s1
PF3D7_0724700	conserved Plasmodium protein	MAL7P1.134.e2.s2
PF3D7_0724700	conserved Plasmodium protein	MAL7P1.134.e2.s3
PF3D7_0724800	kelch protein	MAL7P1.137.e1
PF3D7_0725100	conserved Plasmodium membrane protein	MAL7P1.138.e1.s1
PF3D7_0725100	conserved Plasmodium membrane protein	MAL7P1.138.e1.s2
PF3D7_0725400	conserved Plasmodium protein	MAL7P1.141.e4.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e2.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e3.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e4.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e4.s2
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e5.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e5.s2
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e6.s1
PF3D7_0726400	conserved Plasmodium membrane protein	MAL7P1.146.e6.s2
PF3D7_0727700	conserved Plasmodium protein	PF07.0114.e1.s1
PF3D7_0727900	conserved Plasmodium protein	PF07.0116.e1.s2
PF3D7_0728000	eukaryotic translation initiation factor 2 alpha subunit	PF07.0117.e2
PF3D7_0728100	conserved Plasmodium membrane protein	PF07.0118.s3
PF3D7_0728100	conserved Plasmodium membrane protein	PF07.0118.s6
PF3D7_0729900	dynein heavy chain	MAL7P1.162.e1.s1
PF3D7_0729900	dynein heavy chain	MAL7P1.162.e1.s2
PF3D7_0729900	dynein heavy chain	MAL7P1.162.e2.s2
PF3D7_0729900	dynein heavy chain	MAL7P1.162.e2.s3
PF3D7_0730300	transcription factor with AP2 domain(s) (AP2-L)	PF07.0126.e1.s1
PF3D7_0730300	transcription factor with AP2 domain(s) (AP2-L)	PF07.0126.e1.s2
PF3D7_0730500	conserved Plasmodium protein	MAL7P1.167.e1.s1
PF3D7_0730500	conserved Plasmodium protein	MAL7P1.167.e1.s3
PF3D7_0730800.1; PF3D7_0730800.2	Plasmodium exported protein	MAL7P1.170.e2.s1
PF3D7_0730900	Plasmodium exported protein	MAL7P1.171.e1.s1
PF3D7_0730900	Plasmodium exported protein	MAL7P1.171.e2.s1
PF3D7_0730900	Plasmodium exported protein	MAL7P1.171.e2.s3
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	PF07.0128.e1.s1
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	PF07.0128.e1.s2
PF3D7_0731600	acyl-CoA synthetase (ACS5)	PF07.0129.e1.s1
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0141.e1.s1
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0141.e1.s2
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0141.e1.s3
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0141.e2.s1
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0140.e1.s1
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0140.e2.s1
PF3D7_0800700	surface-associated interspersed protein 8.3 (SURFIN 8.3) (SURF8.3)	MAL8P1.162.e1.s1
PF3D7_0800700	surface-associated interspersed protein 8.3 (SURFIN 8.3) (SURF8.3)	MAL8P1.162.e3.s1
PF3D7_0801000	Plasmodium exported protein (PHISTc)	PF08.0137.e1.s1
PF3D7_0801000	Plasmodium exported protein (PHISTc)	PF08.0137.e2.s1
PF3D7_0801000	Plasmodium exported protein (PHISTc)	PF08.0137.e2.s2
PF3D7_0801500	conserved Plasmodium protein	PF08.0135.e1.s1
PF3D7_0801800	mannose-6-phosphate isomerase	MAL8P1.156
PF3D7_0802000	glutamate dehydrogenase (GDH3)	PF08.0132.e1.s2
PF3D7_0802500	inositol phosphatase	MAL8P1.151.e1.s1
PF3D7_0802500	inositol phosphatase	MAL8P1.151.e2.s1
PF3D7_0803100	conserved Plasmodium protein	PF08.0127.e1.s2
PF3D7_0803800	20S proteasome beta subunit	MAL8P1.142.e2.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0804400	methionine aminopeptidase 1c (MetAP1c)	MAL8P1.140
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e1.s1
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e1.s3
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e1.s4
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e2.s2
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e2.s3
PF3D7_0804500	conserved Plasmodium membrane protein	MAL8P1.139.e3
PF3D7_0804800	peptidyl-prolyl cis-trans isomerase (CYP24)	PF08.0121.e1.s1
PF3D7_0806500	DnaJ protein	PF08.0115.e1.s1
PF3D7_0806600	kinesin-like protein	MAL8P1.132.e2.s1
PF3D7_0806600	kinesin-like protein	MAL8P1.132.e3.s1
PF3D7_0806600	kinesin-like protein	MAL8P1.132.e5.s1
PF3D7_0806800	vacuolar proton translocating ATPase subunit A	PF08.0113.e1.s1
PF3D7_0807500	proteasome subunit alpha	MAL8P1.128.e3.s1
PF3D7_0807800	proteasome subunit alpha type 5	PF08.0109.e2.s1
PF3D7_0807900	tyrosine--tRNA ligase (TyrRS)	MAL8P1.125.e2
PF3D7_0808200	plasmepsin X	PF08.0108.e1.s1
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0107.e1.s1
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0107.e1.s2
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF08.0107.e2.s1
PF3D7_0809200	asparagine-rich antigen Pfa55-14 (pfa55-14)	PF08.0102.e1.s1
PF3D7_0809200	asparagine-rich antigen Pfa55-14 (pfa55-14)	PF08.0102.e1.s2
PF3D7_0811400	conserved protein	MAL8P1.103.e2.s1
PF3D7_0811600	conserved Plasmodium protein	PF08.0091.e2.s1
PF3D7_0812100	conserved Plasmodium protein	PF08.0089.e1.s1
PF3D7_0812100	conserved Plasmodium protein	PF08.0089.e1.s2
PF3D7_0812400	karyopherin alpha (KARalpha)	PF08.0087.e1.s1
PF3D7_0812500	RNA binding protein	PF08.0086.e1.s1
PF3D7_0813400	conserved Plasmodium protein	PF08.0081.e2.s1
PF3D7_0813500	conserved Plasmodium protein	PF08.0080.e1.s1
PF3D7_0813900	40S ribosomal protein S16	PF08.0076.e2.s1
PF3D7_0814000	60S ribosomal protein L13-2	PF08.0075.e2.s1
PF3D7_0814600	conserved Plasmodium protein	PF08.0073.e1.s1
PF3D7_0815400	conserved Plasmodium protein	MAL8P1.85.e1.s1
PF3D7_0816600	ClpB protein (ClpB1)	PF08.0063.e1.s1
PF3D7_0817300	asparagine-rich antigen	PF08.0060.e1.s3
PF3D7_0818200	14-3-3 protein (14-3-3I)	MAL8P1.69.e3
PF3D7_0818500	zinc finger protein	PF08.0056.e2
PF3D7_0818900	heat shock protein 70 (HSP70)	PF08.0054
PF3D7_0819800	conserved Plasmodium protein	MAL8P1.60.e14.s1
PF3D7_0819800	conserved Plasmodium protein	MAL8P1.60.e15.s1
PF3D7_0819800	conserved Plasmodium protein	MAL8P1.60.e16.s1
PF3D7_0819800	conserved Plasmodium protein	MAL8P1.60.e4.s1
PF3D7_0821400	conserved Plasmodium protein	MAL8P1.52.e2.s1
PF3D7_0822600	protein transport protein Sec23 (SEC23)	PF08.0036.e2.s1
PF3D7_0822900	conserved Plasmodium protein	PF08.0035
PF3D7_0823300	histone acetyltransferase GCN5 (GCN5)	PF08.0034.e1.s2
PF3D7_0823300	histone acetyltransferase GCN5 (GCN5)	PF08.0034.e2.s1
PF3D7_0823300	histone acetyltransferase GCN5 (GCN5)	PF08.0034.e3.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0823800	DnaJ protein	PF08.0032
PF3D7_0825900	conserved Plasmodium protein	PF08.0023.e1.s1
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s1
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s2
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s3
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s4
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s7
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s8
PF3D7_0826100	E3 ubiquitin-protein ligase	MAL8P1.23.s9
PF3D7_0826500	ubiquitin conjugation factor E4 B (UBE4B)	PF08.0020.e1.s1
PF3D7_0826500	ubiquitin conjugation factor E4 B (UBE4B)	PF08.0020.e1.s2
PF3D7_0826700	receptor for activated c kinase (RACK)	PF08.0019.e2
PF3D7_0827100	translation initiation factor IF-2	PF08.0018.s1
PF3D7_0827100	translation initiation factor IF-2	PF08.0018.s2
PF3D7_0827800	SET domain protein (SET3)	PF08.0012.e1.s2
PF3D7_0827800	SET domain protein (SET3)	PF08.0012.e1.s3
PF3D7_0827900	protein disulfide isomerase (PDI8)	MAL8P1.17.e2
PF3D7_0829000	conserved Plasmodium membrane protein	MAL8P1.11.e1
PF3D7_0829000	conserved Plasmodium membrane protein	MAL8P1.11.e2.s1
PF3D7_0829000	conserved Plasmodium membrane protein	MAL8P1.11.e2.s2
PF3D7_0829200	prohibitin	PF08.0006.e1.s1
PF3D7_0829500	conserved Plasmodium protein	MAL8P1.7.e1
PF3D7_0829500	conserved Plasmodium protein	MAL8P1.7.e1.s1
PF3D7_0829500	conserved Plasmodium protein	MAL8P1.7.e2
PF3D7_0829600	early transcribed membrane protein 8 (ETRAMP8)	MAL8P1.6.e2
PF3D7_0900200	rifin (RIF)	PFI0010c.e2.s1
PF3D7_0903000	conserved protein	PFI0145w
PF3D7_0903400	DEAD/DEAH box helicase	PFI0165c.e1.s1
PF3D7_0903400	DEAD/DEAH box helicase	PFI0165c.e1.s3
PF3D7_0903400	DEAD/DEAH box helicase	PFI0165c.e2
PF3D7_0903500	conserved Plasmodium protein	PFI0170w.e1.s1
PF3D7_0903500	conserved Plasmodium protein	PFI0170w.e1.s2
PF3D7_0903700	alpha tubulin 1	PFI0180w.e3.s1
PF3D7_0904800	replication protein A1, small fragment	PFI0235w.e2.s1
PF3D7_0904900	Cu2 -transporting ATPase (CUP)	PFI0240c.e1.s1
PF3D7_0904900	Cu2 -transporting ATPase (CUP)	PFI0240c.e1.s2
PF3D7_0904900	Cu2 -transporting ATPase (CUP)	PFI0240c.e1.s3
PF3D7_0905300	dynein heavy chain	PFI0260c.s2
PF3D7_0905300	dynein heavy chain	PFI0260c.s5
PF3D7_0905300	dynein heavy chain	PFI0260c.s6
PF3D7_0905300	dynein heavy chain	PFI0260c.s7
PF3D7_0905400	high molecular weight rhoptry protein 3 (RhopH3)	PFI0265c.e3.s1
PF3D7_0905400	high molecular weight rhoptry protein 3 (RhopH3)	PFI0265c.e6.s1
PF3D7_0905400	high molecular weight rhoptry protein 3 (RhopH3)	PFI0265c.e7.s1
PF3D7_0906400	dynein light intermediate chain 2, cytosolic	PFI0315c.e1.s1
PF3D7_0907100	conserved Plasmodium protein	PFI0340c.s1
PF3D7_0907100	conserved Plasmodium protein	PFI0340c.s2
PF3D7_0907200	GTPase activator	PFI0345w.e1.s1
PF3D7_0907200	GTPase activator	PFI0345w.e1.s2

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0907400	ATP-dependent protease ATPase subunit ClpY (ClpY)	PFI0355c.e1.s1
PF3D7_0907700	subunit of proteaseome activator complex	PFI0370c.e1.s1
PF3D7_0908500	conserved Plasmodium protein	PFI0410c.e15.s1
PF3D7_0908500	conserved Plasmodium protein	PFI0410c.e17.s1
PF3D7_0908500	conserved Plasmodium protein	PFI0410c.e18.s1
PF3D7_0908500	conserved Plasmodium protein	PFI0410c.e2.s1
PF3D7_0908500	conserved Plasmodium protein	PFI0410c.e4.s1
PF3D7_0909500	subpellicular microtubule protein 1 (SPM1)	PFI0460w
PF3D7_0910500	DNA repair protein REV1	PFI0510c.e1.s2
PF3D7_0911100	conserved Plasmodium protein	PFI0540w.e1.s1
PF3D7_0911300	cysteine repeat modular protein 1 (CRMP1)	PFI0550w.e1.s1
PF3D7_0911300	cysteine repeat modular protein 1 (CRMP1)	PFI0550w.e1.s2
PF3D7_0911300	cysteine repeat modular protein 1 (CRMP1)	PFI0550w.e1.s4
PF3D7_0911300	cysteine repeat modular protein 1 (CRMP1)	PFI0550w.e2
PF3D7_0911700	GTP binding protein	PFI0570w
PF3D7_0911900	falstatin (ICP)	PFI0580c.e2
PF3D7_0912900	26S proteasome regulatory subunit	PFI0630w.e1.s1
PF3D7_0912900	26S proteasome regulatory subunit	PFI0630w.e2.s1
PF3D7_0915000	type II NADH:ubiquinone oxidoreductase (NDH2)	PFI0735c.e1.s1
PF3D7_0915700	conserved Plasmodium protein	PFI0770c.e2.s1
PF3D7_0916700	RNA-binding protein musashi (HoMu)	PFI0820c.e1.s1
PF3D7_0917500	conserved Plasmodium protein	PFI0855w.e1.s1
PF3D7_0917900	heat shock protein 70 (HSP70-2)	PFI0875w.e2
PF3D7_0918000	secreted acid phosphatase (GAP50)	PFI0880c.e1.s1
PF3D7_0918300	eukaryotic translation initiation factor 3 subunit 5	PFI0895c.e1.s1
PF3D7_0918300	eukaryotic translation initiation factor 3 subunit 5	PFI0895c.e2.s1
PF3D7_0918900	gamma-glutamylcysteine synthetase (gammaGCS)	PFI0925w.e1.s1
PF3D7_0918900	gamma-glutamylcysteine synthetase (gammaGCS)	PFI0925w.e1.s2
PF3D7_0919400	protein disulfide isomerase (PDI9)	PFI0950w.e1.s1
PF3D7_0920400	conserved Plasmodium protein	PFI1000w.s1
PF3D7_0920400	conserved Plasmodium protein	PFI1000w.s2
PF3D7_0920700	conserved Plasmodium protein	PFI1015w
PF3D7_0920800	inosine-5'-monophosphate dehydrogenase	PFI1020c.e1.s1
PF3D7_0921300	conserved Plasmodium protein	PFI1045w.e2
PF3D7_0921400	Fe-S-cluster redox enzyme (NifU)	PFI1050c.e1
PF3D7_0921600	tetratricopeptide repeat family protein	PFI1060w.e1.s2
PF3D7_0922100	ubiquitin-like protein	PFI1085w.e1.s2
PF3D7_0922200	S-adenosylmethionine synthetase (SAMS)	PFI1090w.e1.s1
PF3D7_0922500	phosphoglycerate kinase (PGK)	PFI1105w
PF3D7_0922600	glutamine synthetase	PFI1110w.e1.s1
PF3D7_0923800.1; PF3D7_0923800.2	thioredoxin reductase (TRXR)	PFI1170c.e1.s1
PF3D7_0925800	conserved Plasmodium protein	PFI1265w.e1.s1
PF3D7_0925800	conserved Plasmodium protein	PFI1265w.e1.s2
PF3D7_0925900	conserved Plasmodium protein	PFI1270w.e2.s1
PF3D7_0927300	fumarate hydratase	PFI1340w.e1.s1
PF3D7_0927400	conserved Plasmodium protein	PFI1345c.e2.s1
PF3D7_0927400	conserved Plasmodium protein	PFI1345c.e3.s1
PF3D7_0929000	conserved Plasmodium protein	PFI1425w.e2

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e1.s1
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e2.s1
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e5.s1
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e6.s1
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e7.s1
PF3D7_0929400	high molecular weight rhoptry protein 2 (RhopH2)	PFI1445w.e9.s1
PF3D7_0930300	merozoite surface protein 1 (MSP1)	PFI1475w.s1
PF3D7_0930300	merozoite surface protein 1 (MSP1)	PFI1475w.s2
PF3D7_0930800	conserved Plasmodium membrane protein	PFI1500w.e2.s2
PF3D7_0930800	conserved Plasmodium membrane protein	PFI1500w.e2.s4
PF3D7_0931100	nucleolar protein Nop52	PFI1510w
PF3D7_0931300	asparagine-rich antigen	PFI1520w
PF3D7_0931800	proteasome precursor	PFI1545c.e1.s1
PF3D7_0932200	profilin (PFN)	PFI1565w.e3.s1
PF3D7_0933500	gamma-tubulin complex component	PFI1620c.s1
PF3D7_0933500	gamma-tubulin complex component	PFI1620c.s2
PF3D7_0933600	mitochondrial-processing peptidase subunit beta (MAS1)	PFI1625c.e1.s1
PF3D7_0934000	histidine--tRNA ligase	PFI1645c.e1.s1
PF3D7_0934500	vacuolar ATP synthase subunit e	PFI1670c.e1.s1
PF3D7_0934800	cAMP-dependent protein kinase catalytic subunit (PKAc)	PFI1685w.e5.s1
PF3D7_0935800	cytoadherence linked asexual protein 9 (CLAG9)	PFI1730w.e1
PF3D7_0935800	cytoadherence linked asexual protein 9 (CLAG9)	PFI1730w.e2
PF3D7_0935800	cytoadherence linked asexual protein 9 (CLAG9)	PFI1730w.e8
PF3D7_0935800	cytoadherence linked asexual protein 9 (CLAG9)	PFI1730w.e9
PF3D7_0935900	ring-exported protein 1 (REX1)	PFI1735c.e1.s1
PF3D7_0935900	ring-exported protein 1 (REX1)	PFI1735c.e2.s1
PF3D7_0936800	Plasmodium exported protein (PHISTc)	PFI1780w.e2.s1
PF3D7_1001000	Plasmodium exported protein (hyp12) (PfJ13)	PF10.0013.e2.s1
PF3D7_1002800	DnaJ protein	PF10.0032.e1.s2
PF3D7_1003500	40S ribosomal protein S20e	PF10.0038.e2.s1
PF3D7_1003600	membrane skeletal protein IMC1-related (ALV5)	PF10.0039.e1.s1
PF3D7_1003800	U5 small nuclear ribonuclear protein	PF10.0041.e1.s1
PF3D7_1003800	U5 small nuclear ribonuclear protein	PF10.0041.e1.s2
PF3D7_1004100	hypothetical protein	PF10.0044.e8
PF3D7_1004200	conserved Plasmodium membrane protein	PF10.0045.e1.s1
PF3D7_1004200	conserved Plasmodium membrane protein	PF10.0045.e2.s1
PF3D7_1004200	conserved Plasmodium membrane protein	PF10.0045.e2.s2
PF3D7_1004200	conserved Plasmodium membrane protein	PF10.0045.e2.s3
PF3D7_1004400	RNA binding protein	PF10.0047.e1.s1
PF3D7_1005100	conserved protein	PF10.0054.e1.s2
PF3D7_1005500	regulator of nonsense transcripts	PF10.0057.e2.s1
PF3D7_1005500	regulator of nonsense transcripts	PF10.0057.e2.s2
PF3D7_1006800	RNA binding protein	PF10.0068.e4.s1
PF3D7_1007700	transcription factor with AP2 domain(s) (ApiAP2)	PF10.0075.e1.s1
PF3D7_1007700	transcription factor with AP2 domain(s) (ApiAP2)	PF10.0075.e1.s2
PF3D7_1008000	inositol polyphosphate kinase (IPK1)	PF10.0078.s1
PF3D7_1008000	inositol polyphosphate kinase (IPK1)	PF10.0078.s2
PF3D7_1008000	inositol polyphosphate kinase (IPK1)	PF10.0078.s3
PF3D7_1008100	conserved Plasmodium protein	PF10.0079.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1008100	conserved Plasmodium protein	PF10.0079.s3
PF3D7_1008100	conserved Plasmodium protein	PF10.0079.s4
PF3D7_1008400	26S proteasome regulatory subunit 4	PF10.0081.e1.s1
PF3D7_1008500	conserved Plasmodium membrane protein	PF10.0082.e3.s1
PF3D7_1008800	nucleolar protein 5 (NOP5)	PF10.0085.e1.s1
PF3D7_1009500	metallopeptidase	PF10.0092.e3
PF3D7_1009800	conserved Plasmodium membrane protein	PF10.0095.e1.s1
PF3D7_1009800	conserved Plasmodium membrane protein	PF10.0095.e1.s2
PF3D7_1010600	eukaryotic translation initiation factor 2 beta subunit	PF10.0103.e1.s1
PF3D7_1011800	QF122 antigen	PF10.0115.e1.s1
PF3D7_1011800	QF122 antigen	PF10.0115.e1.s2
PF3D7_1012600	GMP synthetase (GMPS)	PF10.0123.e1.s1
PF3D7_1012700	protein phosphatase	PF10.0124.e1.s1
PF3D7_1012700	protein phosphatase	PF10.0124.e1.s2
PF3D7_1012800	conserved Plasmodium protein	PF10.0125
PF3D7_1013500	phosphoinositide-specific phospholipase C (PI-PLC)	PF10.0132.e1.s1
PF3D7_1013500	phosphoinositide-specific phospholipase C (PI-PLC)	PF10.0132.e1.s2
PF3D7_1013600	conserved Plasmodium protein	PF10.0133.s1
PF3D7_1013600	conserved Plasmodium protein	PF10.0133.s2
PF3D7_1013600	conserved Plasmodium protein	PF10.0133.s3
PF3D7_1014100	conserved Plasmodium protein	PF10.0138.s1
PF3D7_1014100	conserved Plasmodium protein	PF10.0138.s2
PF3D7_1014300	conserved protein	PF10.0140.e1.s1
PF3D7_1014300	conserved protein	PF10.0140.e1.s2
PF3D7_1014300	conserved protein	PF10.0140.e1.s3
PF3D7_1014300	conserved protein	PF10.0140.e2.s1
PF3D7_1014600	transcriptional coactivator ADA2 (ADA2)	PF10.0143.e1.s1
PF3D7_1014600	transcriptional coactivator ADA2 (ADA2)	PF10.0143.e1.s2
PF3D7_1014600	transcriptional coactivator ADA2 (ADA2)	PF10.0143.e1.s3
PF3D7_1015300	methionine aminopeptidase 1b (MetAP1b)	PF10.0150.e1.s1
PF3D7_1015600	heat shock protein 60 (HSP60)	PF10.0153.e2.s1
PF3D7_1015900	enolase (ENO)	PF10.0155.e2.s1
PF3D7_1016300	glycophorin binding protein (GBP)	PF10.0159.e1.s1
PF3D7_1016500; PF3D7_1016600	Plasmodium exported protein (PHISTc)	PF10.0161.e1.s1
PF3D7_1016500; PF3D7_1016600	Plasmodium exported protein (PHISTc)	PF10.0161.e2.s1
PF3D7_1016900	early transcribed membrane protein 10.3 (ETRAMP10.3)	PF10.0164.e1.s1
PF3D7_1017000	DNA polymerase delta catalytic subunit	PF10.0165.e1.s1
PF3D7_1017000	DNA polymerase delta catalytic subunit	PF10.0165.e1.s2
PF3D7_1017900	26s proteasome regulatory subunit p55	PF10.0174.e2.s1
PF3D7_1018300	conserved Plasmodium protein	PF10.0177.e1.s1
PF3D7_1018300	conserved Plasmodium protein	PF10.0177.e2.s1
PF3D7_1018300	conserved Plasmodium protein	PF10.0177.e2.s2
PF3D7_1018500; PF3D7_1018600	PHF5-like protein; conserved Plasmodium protein	PF10.0179.e1.s1
PF3D7_1019000	eukaryotic translation initiation factor subunit eIF2A	PF10.0183.e1.s1
PF3D7_1019000	eukaryotic translation initiation factor subunit eIF2A	PF10.0183.e1.s2
PF3D7_1019100	conserved Plasmodium protein	PF10.0184.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1019400	60S ribosomal protein L30e	PF10.0187.e2.s1
PF3D7_1020200	conserved Plasmodium protein	PF10.0195.e1.s1
PF3D7_1020200	conserved Plasmodium protein	PF10.0195.e2.s1
PF3D7_1020300	cytoplasmic dynein intermediate chain	PF10.0196.e1.s1
PF3D7_1020700	ribosomal processing ATPase	PF10.0200.e1.s1
PF3D7_1020700	ribosomal processing ATPase	PF10.0200.e1.s2
PF3D7_1021600	deoxyribose-phosphate aldolase	PF10.0210.e1.s1
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e1.s2
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e1.s3
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e2.s1
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e2.s2
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e2.s3
PF3D7_1021700	conserved Plasmodium membrane protein	PF10.0211.e3.s1
PF3D7_1021900	conserved Plasmodium protein (10b antigen)	PF10.0213.e1.s1
PF3D7_1021900	conserved Plasmodium protein (10b antigen)	PF10.0213.e1.s2
PF3D7_1021900	conserved Plasmodium protein (10b antigen)	PF10.0213.e1.s3
PF3D7_1022000	RNA binding protein	PF10.0214.e2
PF3D7_1022000	RNA binding protein	PF10.0214.e3
PF3D7_1022100; PF3D7_1022200	conserved Plasmodium membrane protein	PF10.0215.e1.s1
PF3D7_1022100; PF3D7_1022200	conserved Plasmodium membrane protein	PF10.0215.e2.s1
PF3D7_1022100; PF3D7_1022200	conserved Plasmodium membrane protein	PF10.0215.e4.s1
PF3D7_1022100; PF3D7_1022200	conserved Plasmodium membrane protein	PF10.0215.e5.s1
PF3D7_1023100	dynein heavy chain	PF10.0224.e1.s1
PF3D7_1023100	dynein heavy chain	PF10.0224.e1.s2
PF3D7_1023100	dynein heavy chain	PF10.0224.e1.s3
PF3D7_1023100	dynein heavy chain	PF10.0224.e1.s4
PF3D7_1023100	dynein heavy chain	PF10.0224.e1.s6
PF3D7_1023800	conserved Plasmodium protein	PF10.0231.e2
PF3D7_1023900	chromodomain-helicase-DNA-binding protein 1 homolog (CHD1)	PF10.0232.s1
PF3D7_1023900	chromodomain-helicase-DNA-binding protein 1 homolog (CHD1)	PF10.0232.s3
PF3D7_1023900	chromodomain-helicase-DNA-binding protein 1 homolog (CHD1)	PF10.0232.s4
PF3D7_1024800	conserved Plasmodium protein	PF10.0242.e2.s2
PF3D7_1025000	formin 2	PF10.0244.e4
PF3D7_1025000	formin 2	PF10.0244.e5
PF3D7_1025500	conserved Plasmodium protein	PF10.0251.e2.s2
PF3D7_1025500	conserved Plasmodium protein	PF10.0251.e2.s3
PF3D7_1025500	conserved Plasmodium protein	PF10.0251.e3.s1
PF3D7_1026300	conserved Plasmodium protein	PF10.0260.e2.s1
PF3D7_1026400	cell division cycle protein 20 homolog (CDC20)	PF10.0261
PF3D7_1026800	40S ribosomal protein S2B	PF10.0264.e2.s1
PF3D7_1027800	60S ribosomal protein L3	PF10.0272
PF3D7_1032700	conserved Plasmodium protein	PF10.0319

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1032800; PF3D7_1032900	leucine-rich repeat protein (LRR1); RNA polymerase II-associated protein 1	PF10.0320.e1.s1
PF3D7_1032800; PF3D7_1032900	leucine-rich repeat protein (LRR1); RNA polymerase II-associated protein 1	PF10.0320.e1.s2
PF3D7_1032800; PF3D7_1032900	leucine-rich repeat protein (LRR1); RNA polymerase II-associated protein 1	PF10.0320.e2.s1
PF3D7_1033100	S-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC)	PF10.0322.s1
PF3D7_1033100	S-adenosylmethionine decarboxylase/ornithine decarboxylase (AdoMetDC/ODC)	PF10.0322.s2
PF3D7_1033200	early transcribed membrane protein 10.2 (ETRAMP10.2)	PF10.0323.e1.s1
PF3D7_1033700	bromodomain protein	PF10.0328.e3.s1
PF3D7_1034400	flavoprotein subunit of succinate dehydrogenase (SDHA)	PF10.0334.e1.s1
PF3D7_1034900	methionine--tRNA ligase	PF10.0340.e1.s1
PF3D7_1035200	S-antigen	PF10.0343
PF3D7_1035700	duffy binding-like merozoite surface protein (DBLMSP)	PF10.0348.e1.s1
PF3D7_1036400	liver stage antigen 1 (LSA1)	PF10.0356.e1
PF3D7_1036400	liver stage antigen 1 (LSA1)	PF10.0356.e2.s1
PF3D7_1036400	liver stage antigen 1 (LSA1)	PF10.0356.e2.s2
PF3D7_1036900	conserved Plasmodium protein	PF10.0361.e1.s2
PF3D7_1036900	conserved Plasmodium protein	PF10.0361.e2.s1
PF3D7_1037600	DNA repair helicase rad25	PF10.0369.e2.s1
PF3D7_1037600	DNA repair helicase rad25	PF10.0369.e3.s1
PF3D7_1038400	gametocyte-specific protein (Pf11-1)	PF10.0374.e3.s1
PF3D7_1038400	gametocyte-specific protein (Pf11-1)	PF10.0374.e4.s1
PF3D7_1038400	gametocyte-specific protein (Pf11-1)	PF10.0374.e6.s1
PF3D7_1038400	gametocyte-specific protein (Pf11-1)	PF10.0374.e6.s2
PF3D7_1038900	phospholipase	PF10.0379.e1.s1
PF3D7_1040800	rifin (RIF)	PF10.0401.e2.s1
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF11.0008.e1.s1
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF11.0008.e1.s2
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF11.0008.e1.s3
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF11.0008.e2.s1
PF3D7_1102500	Plasmodium exported protein (PHISTb) (GEXP02)	PF11.0037.e1.s1
PF3D7_1102500	Plasmodium exported protein (PHISTb) (GEXP02)	PF11.0037.e2.s1
PF3D7_1103100	60S acidic ribosomal protein P1	PF11.0043.e1.s1
PF3D7_1103100	60S acidic ribosomal protein P1	PF11.0043.e2.s1
PF3D7_1103700	casein kinase II beta chain (CK2beta1)	PF11.0048.e1.s1
PF3D7_1103800	CCR4-NOT transcription complex subunit 1 (NOT1)	PF11.0049.e1.s1
PF3D7_1103800	CCR4-NOT transcription complex subunit 1 (NOT1)	PF11.0049.e1.s2
PF3D7_1103800	CCR4-NOT transcription complex subunit 1 (NOT1)	PF11.0049.e1.s4
PF3D7_1104200	chromatin remodeling protein (SNF2L)	PF11.0053.e1.s2
PF3D7_1104400	conserved protein	PF11.0055.e1.s1
PF3D7_1104400	conserved protein	PF11.0055.e2.s1
PF3D7_1105400	40S ribosomal protein S4	PF11.0065.e2.s1
PF3D7_1105600	translocon component PTEX88 (PTEX88)	PF11.0067.e1.s1
PF3D7_1105700	conserved protein	PF11.0068.e1.s1
PF3D7_1105800	conserved Plasmodium protein	PF11.0069.e1.s1
PF3D7_1107100	nucleic acid binding protein	PF11.0083.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1107100	nucleic acid binding protein	PF11.0083.e2.s1
PF3D7_1107100	nucleic acid binding protein	PF11.0083.e6.s1
PF3D7_1107300	polyadenylate-binding protein-interacting protein 1 (PAIP1)	PF11.0086.s1
PF3D7_1107300	polyadenylate-binding protein-interacting protein 1 (PAIP1)	PF11.0086.s2
PF3D7_1107300	polyadenylate-binding protein-interacting protein 1 (PAIP1)	PF11.0086.s3
PF3D7_1107300	polyadenylate-binding protein-interacting protein 1 (PAIP1)	PF11.0086.s4
PF3D7_1107800	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0091.e1.s1
PF3D7_1107800	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0091.e1.s2
PF3D7_1107800	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0091.e2.s1
PF3D7_1108400	casein kinase 2, alpha subunit (CK2alpha)	PF11.0096.e1.s1
PF3D7_1108500	succinyl-CoA synthetase alpha subunit	PF11.0097.e2
PF3D7_1108700	heat shock protein DnaJ homologue Pfj2	PF11.0099.e1.s1
PF3D7_1109900	apicoplast ribosomal protein L36e precursor	PF11.0106
PF3D7_1110200	pre-mRNA-processing factor 6 (PRPF6)	PF11.0108.e1.s1
PF3D7_1110200	pre-mRNA-processing factor 6 (PRPF6)	PF11.0108.e1.s2
PF3D7_1110200	pre-mRNA-processing factor 6 (PRPF6)	PF11.0108.e2.s1
PF3D7_1110400	asparagine-rich antigen	PF11.0111.e3.s1
PF3D7_1110400	asparagine-rich antigen	PF11.0111.e3.s2
PF3D7_1110700	actin-like protein (ALP1)	PF11.0114.e2.s1
PF3D7_1110700	actin-like protein (ALP1)	PF11.0114.e3.s1
PF3D7_1112300	conserved Plasmodium protein	PF11.0129.e1
PF3D7_1112700	conserved Plasmodium protein	PF11.0527.e3.s1
PF3D7_1113100	protein tyrosine phosphatase (PRL)	PF11.0139
PF3D7_1113700	glyoxalase I (GloI)	PF11.0145.e1.s1
PF3D7_1113900	mitogen-activated protein kinase 2 (MAP2)	PF11.0147.e1.s1
PF3D7_1114700	serine/threonine protein kinase (CLK3)	PF11.0156.e1
PF3D7_1114900	conserved Plasmodium protein	PF11.0158.e1.s1
PF3D7_1114900	conserved Plasmodium protein	PF11.0158.e1.s2
PF3D7_1115300	cysteine proteinase falcipain 2b (FP2B)	PF11.0161.e1.s1
PF3D7_1115400	cysteine proteinase falcipain 3 (FP3)	PF11.0162.e1.s1
PF3D7_1115600	peptidyl-prolyl cis-trans isomerase (CYP19B)	PF11.0164.e3.s1
PF3D7_1115700	cysteine proteinase falcipain 2a (FP2A)	PF11.0165.e1.s1
PF3D7_1116700	cathepsin C, homolog,dipeptidyl peptidase 1 (DPAP1)	PF11.0174.e1.s1
PF3D7_1116800	heat shock protein 101 (HSP101)	PF11.0175.e4.s1
PF3D7_1117700	GTP-binding nuclear protein ran/tc4 (RAN)	PF11.0183.e2
PF3D7_1118200	heat shock protein 90	PF11.0188.e1.s1
PF3D7_1118500	box C/D snoRNP rRNA 2'-O-methylation factor	PF11.0191
PF3D7_1118600	histone acetyltransferase (MYST)	PF11.0192
PF3D7_1120100	phosphoglycerate mutase (PGM1)	PF11.0208.e3
PF3D7_1120500	tRNA nucleotidyltransferase	PF11.0212.e1.s1
PF3D7_1120600	conserved Plasmodium protein	PF11.0213.e1.s2
PF3D7_1120600	conserved Plasmodium protein	PF11.0213.e1.s3
PF3D7_1121700	protein GCN20 (GCN20)	PF11.0225.e1.s1
PF3D7_1121800	petidase, M16 family	PF11.0226.s1
PF3D7_1121800	petidase, M16 family	PF11.0226.s2
PF3D7_1121800	petidase, M16 family	PF11.0226.s3
PF3D7_1122400	conserved Plasmodium protein	PF11.0232.e1.s1
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	PF11.0239.e1.s1
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	PF11.0239.e1.s2

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	PF11.0239.e2
PF3D7_1122900	dynein heavy chain	PF11.0240.e1.s1
PF3D7_1122900	dynein heavy chain	PF11.0240.e2.s1
PF3D7_1122900	dynein heavy chain	PF11.0240.e2.s2
PF3D7_1122900	dynein heavy chain	PF11.0240.e2.s3
PF3D7_1122900	dynein heavy chain	PF11.0240.e2.s5
PF3D7_1123100	calcium-dependent protein kinase 7 (CDPK7)	PF11.0242.e2.s1
PF3D7_1123400	translation elongation factor EF-1, subunit alpha	PF11.0245.e1
PF3D7_1123400	translation elongation factor EF-1, subunit alpha	PF11.0245.e2
PF3D7_1123600	RAP protein	PF11.0247
PF3D7_1123900	splicing factor	PF11.0250.e3.s1
PF3D7_1124600	ethanolamine kinase (EK)	PF11.0257.e1.s1
PF3D7_1124700	GrpE protein homolog, mitochondrial (MGE1)	PF11.0258.e1.s1
PF3D7_1124800	nuclear preribosomal assembly protein	PF11.0259.e2.s1
PF3D7_1125500	small nuclear ribonucleoprotein D1 (SNRPD1)	PF11.0266.e1.s1
PF3D7_1126000	threonine--tRNA ligase (ThrRS)	PF11.0270.e1.s1
PF3D7_1126000	threonine--tRNA ligase (ThrRS)	PF11.0270.e1.s2
PF3D7_1126200	40S ribosomal protein S18	PF11.0272.e1.s1
PF3D7_1126300	DnaJ protein	PF11.0273.e2
PF3D7_1127000	protein phosphatase	PF11.0281.e6.s1
PF3D7_1128300	6-phosphofructokinase (PFK11)	PF11.0294.e1.s1
PF3D7_1128300	6-phosphofructokinase (PFK11)	PF11.0294.e2.s1
PF3D7_1128300	6-phosphofructokinase (PFK11)	PF11.0294.e3.s1
PF3D7_1128900	conserved Plasmodium protein	PF11.0300.e1.s1
PF3D7_1128900	conserved Plasmodium protein	PF11.0300.e2.s1
PF3D7_1129000	spermidine synthase (SpdSyn)	PF11.0301.e2.s1
PF3D7_1129100	parasitophorous vacuolar protein 1 (PV1)	PF11.0302.e1.s1
PF3D7_1129600	phosphatidylinositol-4-phosphate-5-kinase	PF11.0307.e1.s1
PF3D7_1129600	phosphatidylinositol-4-phosphate-5-kinase	PF11.0307.e1.s2
PF3D7_1130200	60S ribosomal protein P0 (PfP0)	PF11.0313
PF3D7_1131400	conserved Plasmodium protein	PF11.0324.e1.s1
PF3D7_1131600	conserved Plasmodium protein	PF11.0326.s2
PF3D7_1131900	conserved Plasmodium protein	PF11.0328
PF3D7_1132400	conserved Plasmodium membrane protein	PF11.0333.e2.s1
PF3D7_1132400	conserved Plasmodium membrane protein	PF11.0333.e2.s2
PF3D7_1133100	conserved membrane protein	PF11.0341.e1.s1
PF3D7_1133100	conserved membrane protein	PF11.0341.e1.s2
PF3D7_1133200	conserved Plasmodium protein	PF11.0342.e1.s2
PF3D7_1133200	conserved Plasmodium protein	PF11.0342.e2.s1
PF3D7_1133400	apical membrane antigen 1 (AMA1)	PF11.0344
PF3D7_1134000	heat shock protein 70 (Hsp70-3)	PF11.0351.e1.s1
PF3D7_1134100	protein disulfide isomerase (PDI-11)	PF11.0352.e1.s1
PF3D7_1134200	conserved Plasmodium protein	PF11.0353.s1
PF3D7_1134500	alpha/beta hydrolase	PF11.0356.s1
PF3D7_1134500	alpha/beta hydrolase	PF11.0356.s2
PF3D7_1134700	DNA-directed RNA polymerase 1, subunit 2	PF11.0358.e1
PF3D7_1134700	DNA-directed RNA polymerase 1, subunit 2	PF11.0358.e2.s1
PF3D7_1134700	DNA-directed RNA polymerase 1, subunit 2	PF11.0358.e2.s2
PF3D7_1135400	conserved Plasmodium protein	PF11.0364.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1135600	conserved Plasmodium protein	PF11.0368.e1.s1
PF3D7_1135600	conserved Plasmodium protein	PF11.0368.e1.s2
PF3D7_1136300	tudor staphylococcal nuclease (TSN)	PF11.0374.e2.s1
PF3D7_1136300	tudor staphylococcal nuclease (TSN)	PF11.0374.e2.s2
PF3D7_1136500.1; PF3D7_1136500.2	casein kinase 1 (CK1)	PF11.0377.e6.s1
PF3D7_1136900	subtilisin-like protease 2 (SUB2)	PF11.0381.e1.s1
PF3D7_1136900	subtilisin-like protease 2 (SUB2)	PF11.0381.e2.s1
PF3D7_1137100	mitochondrial ribosomal protein S9 precursor	PF11.0382
PF3D7_1138400	guanylyl cyclase (GCalpha)	PF11.0395.e1.s1
PF3D7_1138400	guanylyl cyclase (GCalpha)	PF11.0395.e1.s2
PF3D7_1138400	guanylyl cyclase (GCalpha)	PF11.0395.e1.s3
PF3D7_1138400	guanylyl cyclase (GCalpha)	PF11.0395.e1.s4
PF3D7_1138400	guanylyl cyclase (GCalpha)	PF11.0395.e1.s5
PF3D7_1139300	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0404.e2.s1
PF3D7_1139300	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0404.e2.s2
PF3D7_1139300	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0404.e2.s3
PF3D7_1139300	transcription factor with AP2 domain(s) (ApiAP2)	PF11.0404.e3.s1
PF3D7_1139500.1; PF3D7_1139500.2	AAA family ATPase	PF11.0405.e1.s1
PF3D7_1139500.1; PF3D7_1139500.2	AAA family ATPase	PF11.0405.e2.s1
PF3D7_1139700	adrenodoxin reductase	PF11.0407.e1.s1
PF3D7_1140500	myosin F (MyoF)	PF11.0416.e1.s1
PF3D7_1140500	myosin F (MyoF)	PF11.0416.e1.s2
PF3D7_1140700	conserved Plasmodium protein	PF11.0418.e1.s1
PF3D7_1140700	conserved Plasmodium protein	PF11.0418.e1.s2
PF3D7_1140700	conserved Plasmodium protein	PF11.0418.e1.s3
PF3D7_1141100	conserved Plasmodium protein	PF11.0422.e1.s2
PF3D7_1142100	conserved Plasmodium protein	PF11.0433.e1.s1
PF3D7_1142100	conserved Plasmodium protein	PF11.0433.e1.s3
PF3D7_1142300	conserved Plasmodium membrane protein	PF11.0435.e2.s1
PF3D7_1142600	60S ribosomal protein L35ae	PF11.0438.e2.s1
PF3D7_1142800	conserved Plasmodium protein	PF11.0440
PF3D7_1142900	conserved Plasmodium protein	PF11.0441.e1
PF3D7_1142900	conserved Plasmodium protein	PF11.0441.e2
PF3D7_1145100	coatamer gamma subunit	PF11.0463
PF3D7_1145400	dynamin-like protein (DYN1)	PF11.0465.e1.s1
PF3D7_1146800	conserved Plasmodium protein	PF11.0479.e2
PF3D7_1146800	conserved Plasmodium protein	PF11.0479.e3.s1
PF3D7_1147000	sporozoite asparagine-rich protein (SLARP)	PF11.0480.e1.s1
PF3D7_1147000	sporozoite asparagine-rich protein (SLARP)	PF11.0480.e1.s3
PF3D7_1147300	conserved Plasmodium protein	PF11.0482.e1.s2
PF3D7_1147800.1; PF3D7_1147800.2	merozoite adhesive erythrocytic binding protein (MAEBL)	PF11.0486.e2.s1
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	PF11.0506.e1.s1
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	PF11.0507.e1.s1
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	PF11.0507.e1.s2
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	PF11.0507.e1.s4

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	PF11.0507.e1.s6
PF3D7_1149200	ring-infected erythrocyte surface antigen	PF11.0509.e2.s1
PF3D7_1149200	ring-infected erythrocyte surface antigen	PF11.0509.e2.s2
PF3D7_1149500	ring-infected erythrocyte surface antigen 2, pseudogene (RESA2)	PF11.0512.e2
PF3D7_1149500	ring-infected erythrocyte surface antigen 2, pseudogene (RESA2)	PF11.0512.e4
PF3D7_1200300	rifin (RIF)	PFL0015c.e2.s1
PF3D7_1201300	liver stage associated protein 1 (LSAP1)	PFL0065w
PF3D7_1201700	conserved Plasmodium membrane protein	PFL0085c.e2
PF3D7_1202200	mitochondrial phosphate carrier protein (MPC)	PFL0110c
PF3D7_1202300	dynein heavy chain	PFL0115w.e14.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e20.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e21.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e22.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e24.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e25.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e29.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e3.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e3.s2
PF3D7_1202300	dynein heavy chain	PFL0115w.e30.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e31.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e33.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e34.s1
PF3D7_1202300	dynein heavy chain	PFL0115w.e9.s1
PF3D7_1202600	conserved protein	PFL0130c.e1
PF3D7_1202600	conserved protein	PFL0130c.e2.s1
PF3D7_1203700	nucleosome assembly protein (NAPL)	PFL0185c.e3
PF3D7_1203900	ubiquitin conjugating enzyme E2 (UBC)	PFL0190w.e4.s1
PF3D7_1204300	eukaryotic translation initiation factor 5A (EIF5A)	PFL0210c
PF3D7_1205500	zinc finger protein	PFL0275w.e1.s1
PF3D7_1205500	zinc finger protein	PFL0275w.e1.s2
PF3D7_1205600	conserved Plasmodium protein	PFL0280c.e6.s1
PF3D7_1205900	conserved protein	PFL0295c.e1.s1
PF3D7_1205900	conserved protein	PFL0295c.e1.s2
PF3D7_1206000	shewanella-like protein phosphatase 2 (SHLP2)	PFL0300c
PF3D7_1206100	IMP-specific 5'-nucleotidase,haloacid dehalogenase hydrolase	PFL0305c.e2.s1
PF3D7_1206200	eukaryotic translation initiation factor 3 subunit 8	PFL0310c.e2
PF3D7_1207000	conserved Plasmodium protein	PFL0350c.e3.s1
PF3D7_1207500	conserved Plasmodium protein	PFL0375w.e2.s1
PF3D7_1208100	conserved Plasmodium protein	PFL0405w.e2.s1
PF3D7_1208100	conserved Plasmodium protein	PFL0405w.e2.s2
PF3D7_1208100	conserved Plasmodium protein	PFL0405w.e2.s4
PF3D7_1208100	conserved Plasmodium protein	PFL0405w.e3.s1
PF3D7_1208100	conserved Plasmodium protein	PFL0405w.e3.s2
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	PFL0410w.e1.s1
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	PFL0410w.e3.s1
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	PFL0410w.e3.s2
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	PFL0410w.e3.s3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1208800	zinc finger protein	PFL0440c.e1.s1
PF3D7_1208800	zinc finger protein	PFL0440c.e1.s2
PF3D7_1208900	conserved Plasmodium protein	PFL0445w.s1
PF3D7_1208900	conserved Plasmodium protein	PFL0445w.s2
PF3D7_1209300	zinc finger transcription factor (KROX1)	PFL0465c.e1.s1
PF3D7_1209400	conserved Plasmodium protein	PFL0470w.e1.s1
PF3D7_1211000	kinesin-like protein	PFL0545w.e3.s1
PF3D7_1211000	kinesin-like protein	PFL0545w.e3.s2
PF3D7_1211200	conserved Plasmodium protein	PFL0555c.e1.s1
PF3D7_1211200	conserved Plasmodium protein	PFL0555c.e1.s2
PF3D7_1211300	DNA helicase MCM8 (MCM8)	PFL0560c.e2.s1
PF3D7_1211400	heat shock protein DNAJ homologue Pfj4 (PfJ4)	PFL0565w.e3.s1
PF3D7_1211700	DNA replication licensing factor MCM5 (MCM5)	PFL0580w.e1.s1
PF3D7_1211900	non-SERCA-type Ca2 -transporting P-ATPase (ATP4)	PFL0590c.e1.s1
PF3D7_1212000	glutathione peroxidase (Trx-G1)	PFL0595c.e1.s1
PF3D7_1212000	glutathione peroxidase (Trx-G1)	PFL0595c.e3.s1
PF3D7_1212700	eukaryotic translation initiation factor 3 subunit 10	PFL0625c.s1
PF3D7_1212900	bromodomain protein	PFL0635c.e1.s1
PF3D7_1212900	bromodomain protein	PFL0635c.e1.s2
PF3D7_1213400	conserved Plasmodium protein	PFL0650c.e1.s1
PF3D7_1213800	proline--tRNA ligase	PFL0670c.e1.s1
PF3D7_1214100	phosphatidylinositol-glycan biosynthesis class O protein	PFL0685w.e2
PF3D7_1214100	phosphatidylinositol-glycan biosynthesis class O protein	PFL0685w.e4
PF3D7_1215000	thioredoxin peroxidase 2 (Trx-Px2)	PFL0725w.e1.s1
PF3D7_1215100	conserved Plasmodium protein	PFL0730w.e1.s1
PF3D7_1216200	glycerol-3-phosphate dehydrogenase	PFL0780w.e4.s1
PF3D7_1216500	male development gene 1 (MDV1)	PFL0795c.e1.s1
PF3D7_1216600	cell traversal protein for ookinetes and sporozoites (CelTOS)	PFL0800c
PF3D7_1216900	DNA-binding chaperone	PFL0815w.e1.s1
PF3D7_1217200	snoRNA-associated small subunit rRNA processing protein	PFL0830w.e1.s1
PF3D7_1218100	conserved Plasmodium protein	PFL0875w.e1
PF3D7_1218200	conserved Plasmodium protein	PFL0880c.e1.s1
PF3D7_1218200	conserved Plasmodium protein	PFL0880c.e1.s2
PF3D7_1218200	conserved Plasmodium protein	PFL0880c.e1.s3
PF3D7_1218300	adaptor protein subunit	PFL0885w.e1.s1
PF3D7_1218600	arginine--tRNA ligase	PFL0900c.e2.s1
PF3D7_1219100	clathrin heavy chain	PFL0930w.e1.s1
PF3D7_1219700	raf kinase inhibitor (RKIP)	PFL0955c.e1.s1
PF3D7_1220000	conserved Plasmodium protein	PFL0965c.e1.s1
PF3D7_1221000	histone-lysine N-methyltransferase, H3 lysine-4 specific (SET10)	PFL1010c.e1.s1
PF3D7_1221000	histone-lysine N-methyltransferase, H3 lysine-4 specific (SET10)	PFL1010c.e1.s2
PF3D7_1221000	histone-lysine N-methyltransferase, H3 lysine-4 specific (SET10)	PFL1010c.e1.s3
PF3D7_1221400	inner membrane complex protein 1h (IMC1h)	PFL1030w.e1.s1
PF3D7_1221700	FbpA domain protein	PFL1045w.e1
PF3D7_1222300	endoplasmin (GRP94)	PFL1070c.e1.s1
PF3D7_1222400	transcription factor with AP2 domain(s) (ApiAP2)	PFL1075w.e1.s2

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1222600	transcription factor with AP2 domain(s) (ApiAP2)	PFL1085w.e1.s1
PF3D7_1222600	transcription factor with AP2 domain(s) (ApiAP2)	PFL1085w.e1.s2
PF3D7_1223500	conserved Plasmodium protein	PFL1130c.e1.s5
PF3D7_1223600	conserved Plasmodium protein	PFL1135c.e1
PF3D7_1223600	conserved Plasmodium protein	PFL1135c.e2.s1
PF3D7_1223600	conserved Plasmodium protein	PFL1135c.e2.s2
PF3D7_1225600	conserved Plasmodium protein	PFL1235c.e2.s1
PF3D7_1225800	ubiquitin-activating enzyme E1 (UBA1)	PFL1245w.e2.s1
PF3D7_1225800	ubiquitin-activating enzyme E1 (UBA1)	PFL1245w.e2.s2
PF3D7_1226000	conserved Plasmodium protein	PFL1255c.e1
PF3D7_1226900	conserved Plasmodium protein	PFL1300c.e1.s1
PF3D7_1227100	DNA helicase 60 (DH60)	PFL1310c.e1.s1
PF3D7_1227300	conserved Plasmodium protein	PFL1320w.e1.s1
PF3D7_1227300	conserved Plasmodium protein	PFL1320w.e1.s2
PF3D7_1228400	conserved Plasmodium protein	PFL1375w.s1
PF3D7_1228400	conserved Plasmodium protein	PFL1375w.s2
PF3D7_1228600	merozoite surface protein 9 (MSP9)	PFL1385c.e1.s1
PF3D7_1228800	conserved Plasmodium protein	PFL1395c.e1.s1
PF3D7_1228800	conserved Plasmodium protein	PFL1395c.e1.s3
PF3D7_1228800	conserved Plasmodium protein	PFL1395c.e1.s4
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	PFL1410c.s1
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	PFL1410c.s3
PF3D7_1229400	macrophage migration inhibitory factor (MIF)	PFL1420w.e2.s1
PF3D7_1229500	T-complex protein 1, gamma subunit	PFL1425w.e2.s1
PF3D7_1232100	60 kDa chaperonin (CPN60)	PFL1545c.e1
PF3D7_1232100	60 kDa chaperonin (CPN60)	PFL1545c.e2
PF3D7_1233300	pentatricopeptide repeat protein	PFL1605w.s1
PF3D7_1233300	pentatricopeptide repeat protein	PFL1605w.s2
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	PFL1620w.e1.s1
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	PFL1620w.e1.s2
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	PFL1620w.e1.s3
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	PFL1620w.e1.s4
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	PFL1620w.e1.s5
PF3D7_1234800	Splicing factor 3B subunit 3 (SF3B3)	PFL1680w.e1.s1
PF3D7_1234800	Splicing factor 3B subunit 3 (SF3B3)	PFL1680w.e1.s2
PF3D7_1235300	CCR4-NOT transcription complex subunit 4 (NOT4)	PFL1705w.s1
PF3D7_1235300	CCR4-NOT transcription complex subunit 4 (NOT4)	PFL1705w.s2
PF3D7_1235400	tetQ family GTPase	PFL1710c.e1.s2
PF3D7_1235600	serine hydroxymethyltransferase (SHMT)	PFL1720w.e3.s1
PF3D7_1235700	ATP synthase subunit beta, mitochondrial	PFL1725w.e1.s1
PF3D7_1236100	clustered-asparagine-rich protein	PFL1745c.e2.s1
PF3D7_1237400	conserved Plasmodium protein	PFL1810w.e2
PF3D7_1237400	conserved Plasmodium protein	PFL1810w.e3
PF3D7_1238100	calcyclin binding protein	PFL1845c.e1
PF3D7_1238800	acyl-CoA synthetase (ACS11)	PFL1880w.e1.s1
PF3D7_1239200	transcription factor with AP2 domain(s) (ApiAP2)	PFL1900w.e1.s2
PF3D7_1239700	cell division protein FtsH	PFL1925w.e1.s1
PF3D7_1239800	conserved Plasmodium protein	PFL1930w.s3
PF3D7_1239800	conserved Plasmodium protein	PFL1930w.s5

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1241700	replication factor C subunit 4	PFL2005w.e2.s1
PF3D7_1242700	40S ribosomal protein S17	PFL2055w.e2
PF3D7_1242800	rab specific GDP dissociation inhibitor (rabGDI)	PFL2060c.e4.s1
PF3D7_1244100	N-alpha-acetyltransferase 15, NatA auxiliary subunit	PFL2120w.e1.s2
PF3D7_1244100	N-alpha-acetyltransferase 15, NatA auxiliary subunit	PFL2120w.e3.s1
PF3D7_1244600	ADP-ribosylation factor GTPase-activating protein (ARFGAP)	PFL2140c.e1.s1
PF3D7_1245100	kinesin-13 (KLP8)	PFL2165w.e2.s1
PF3D7_1245600	kinesin	PFL2190c.e1.s2
PF3D7_1246200	actin I (ACT1)	PFL2215w
PF3D7_1247400	FK506-binding protein (FKBP)-type peptidyl-prolyl isomerase (FKBP35)	PFL2275c.e1.s1
PF3D7_1247800	dipeptidyl peptidase 2 (DPAP2)	PFL2290w.e4.s1
PF3D7_1247800	dipeptidyl peptidase 2 (DPAP2)	PFL2290w.e6.s1
PF3D7_1248700	conserved Plasmodium protein	PFL2335w.s2
PF3D7_1249100	conserved Plasmodium protein	PFL2355w.e2
PF3D7_1249800	conserved Plasmodium protein	PFL2390c.e1.s2
PF3D7_1249800	conserved Plasmodium protein	PFL2390c.e1.s3
PF3D7_1250200	conserved Plasmodium membrane protein	PFL2410w.e1
PF3D7_1250600	eukaryotic translation initiation factor 2b, subunit 2	PFL2430c
PF3D7_1250800	DNA repair protein rhp16	PFL2440w.e2.s1
PF3D7_1250800	DNA repair protein rhp16	PFL2440w.e2.s2
PF3D7_1250900	conserved Plasmodium protein	PFL2445c.e1.s1
PF3D7_1251000	conserved Plasmodium protein	PFL2450c.e1.s1
PF3D7_1251200	coronin	PFL2460w.e3.s1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	PFL2505c.e5.s1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	PFL2505c.e6.s1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	PFL2505c.e7.s1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	PFL2505c.e8.s1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	PFL2505c.e8.s2
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	PFL2520w.e2.s1
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	PFL2520w.e3.s1
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	PFL2520w.e4.s1
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	PFL2520w.e4.s2
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	PFL2520w.e4.s3
PF3D7_1254100	stevor	PFL2610w.e2.s1
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF13.0003.e1.s1
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF13.0003.e1.s2
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	PF13.0003.e2.s1
PF3D7_1302100	gamete antigen 27/25 (Pfg27)	PF13.0011.e1.s1
PF3D7_1302200	early transcribed membrane protein 13 (ETRAMP13)	PF13.0012
PF3D7_1302800	40S ribosomal protein S7	PF13.0014.e1
PF3D7_1303100	methyltransferase-like protein	PF13.0016.e2
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s1
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s5
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s6
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s7
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s8
PF3D7_1303800	conserved Plasmodium protein	MAL13P1.19.e1.s9
PF3D7_1304100	DNA ligase I (LigI)	MAL13P1.22.e1.s1
PF3D7_1304100	DNA ligase I (LigI)	MAL13P1.22.e2.s1
PF3D7_1304500	small heat shock protein	PF13.0021.e1.s1
PF3D7_1306400	26S proteasome regulatory subunit	PF13.0033
PF3D7_1306500	MORN repeat protein	MAL13P1.32.e1.s1
PF3D7_1306500	MORN repeat protein	MAL13P1.32.e3.s1
PF3D7_1306500	MORN repeat protein	MAL13P1.32.e3.s3
PF3D7_1306900	U1 small nuclear ribonucleoprotein a	MAL13P1.35.e1
PF3D7_1307600	DNA-directed RNA polymerase alpha chain	PF13.0040.e2
PF3D7_1308200	carbamoyl phosphate synthetase (cpsSII)	PF13.0044.e2.s1
PF3D7_1308200	carbamoyl phosphate synthetase (cpsSII)	PF13.0044.e2.s2
PF3D7_1308400	conserved Plasmodium protein	MAL13P1.39.e1.s1
PF3D7_1308400	conserved Plasmodium protein	MAL13P1.39.e1.s2
PF3D7_1308400	conserved Plasmodium protein	MAL13P1.39.e2.s1
PF3D7_1308400	conserved Plasmodium protein	MAL13P1.39.e4.s1
PF3D7_1310000	mitochondrial ATP synthase delta subunit (OSCP)	MAL13P1.47.e1.s1
PF3D7_1311400	clathrin-adaptor medium chain	PF13.0062.e1.s1
PF3D7_1311500	26S proteasome regulatory subunit 7	PF13.0063.e2.s1
PF3D7_1311800	M1-family alanyl aminopeptidase (M1AAP)	MAL13P1.56.e1.s1
PF3D7_1311900	vacuolar ATP synthase subunit a (vapA)	PF13.0065.e1.s1
PF3D7_1313600	conserved Plasmodium protein	PF13.0079.e1.s2
PF3D7_1314200	telomerase reverse transcriptase (TERT)	PF13.0080.e1.s1
PF3D7_1314200	telomerase reverse transcriptase (TERT)	PF13.0080.e1.s3
PF3D7_1315200	conserved Plasmodium protein	MAL13P1.78.e1.s2
PF3D7_1317300	conserved Plasmodium protein	PF13.0098.e5.s1
PF3D7_1318300	conserved Plasmodium protein	PF13.0101.e1.s1
PF3D7_1319900	conserved Plasmodium protein	MAL13P1.107.s1
PF3D7_1319900	conserved Plasmodium protein	MAL13P1.107.s2
PF3D7_1320000	rhoptry protein 2 (PRP2)	PF13.0116.e1.s1
PF3D7_1320800	dihydrolipamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex	PF13.0121.e3
PF3D7_1321100	conserved Plasmodium protein	MAL13P1.114.e3.s1
PF3D7_1321100	conserved Plasmodium protein	MAL13P1.114.e3.s3
PF3D7_1321100	conserved Plasmodium protein	MAL13P1.114.e4.s1
PF3D7_1321300	conserved Plasmodium membrane protein	MAL13P1.116.e1.s1
PF3D7_1321300	conserved Plasmodium membrane protein	MAL13P1.116.e1.s2
PF3D7_1321300	conserved Plasmodium membrane protein	MAL13P1.116.e1.s3
PF3D7_1321300	conserved Plasmodium membrane protein	MAL13P1.116.e1.s4
PF3D7_1321900	conserved Plasmodium protein	PF13.0125
PF3D7_1322200	conserved Plasmodium protein	MAL13P1.123.e1.s1
PF3D7_1322200	conserved Plasmodium protein	MAL13P1.123.e1.s2
PF3D7_1322200	conserved Plasmodium protein	MAL13P1.123.e4.s1
PF3D7_1322300	translation initiation factor EIF-2B subunit related	PF13.0126.e3.s2
PF3D7_1322300	translation initiation factor EIF-2B subunit related	PF13.0126.e4.s1
PF3D7_1322400	conserved Plasmodium protein	MAL13P1.125.e3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1323100	60S ribosomal protein L6	PF13.0129.e2.s1
PF3D7_1323600	conserved Plasmodium protein	PF13.0134.e1.s1
PF3D7_1324300	conserved Plasmodium membrane protein	MAL13P1.133.e1.s5
PF3D7_1324300	conserved Plasmodium membrane protein	MAL13P1.133.e1.s6
PF3D7_1324300	conserved Plasmodium membrane protein	MAL13P1.133.e3
PF3D7_1324900	L-lactate dehydrogenase (LDH)	PF13.0141.e1.s1
PF3D7_1325100	phosphoribosylpyrophosphate synthetase	PF13.0143.e1.s1
PF3D7_1325200	oxidoreductase	PF13.0144.e2.s1
PF3D7_1325900	conserved Plasmodium protein	MAL13P1.140.e1.s1
PF3D7_1325900	conserved Plasmodium protein	MAL13P1.140.e1.s2
PF3D7_1325900	conserved Plasmodium protein	MAL13P1.140.e1.s3
PF3D7_1326600	conserved Plasmodium protein	PF13.0148.e1.s5
PF3D7_1326600	conserved Plasmodium protein	PF13.0148.e2.s1
PF3D7_1327300	conserved Plasmodium protein	PF13.0161.e1.s1
PF3D7_1327300	conserved Plasmodium protein	PF13.0161.e1.s2
PF3D7_1327800	ribose-phosphate pyrophosphokinase	PF13.0157.e1.s1
PF3D7_1328100	proteasome subunit beta type 7 precursor	PF13.0156.e1.s1
PF3D7_1328500	alpha/beta-hydrolase	PF13.0153.e1.s1
PF3D7_1328500	alpha/beta-hydrolase	PF13.0153.e1.s2
PF3D7_1329000	DNA-directed RNA polymerase 3 largest subunit	PF13.0150.e1.s1
PF3D7_1329000	DNA-directed RNA polymerase 3 largest subunit	PF13.0150.e1.s2
PF3D7_1329000	DNA-directed RNA polymerase 3 largest subunit	PF13.0150.e2.s1
PF3D7_1329100	myosin C (MyoC)	MAL13P1.148.e3.s2
PF3D7_1329100	myosin C (MyoC)	MAL13P1.148.e3.s3
PF3D7_1330800	conserved Plasmodium protein	PF13.0165.e2.s1
PF3D7_1332200	conserved Plasmodium protein	PF13.0173.e1.s1
PF3D7_1332900	isoleucine--tRNA ligase	PF13.0179.e1.s1
PF3D7_1333000	20 kDa chaperonin (CPN20)	PF13.0180.e6.s1
PF3D7_1333200	ubiquitin-activating enzyme (UBA1)	PF13.0182.s1
PF3D7_1333200	ubiquitin-activating enzyme (UBA1)	PF13.0182.s2
PF3D7_1333800	conserved Plasmodium protein	PF13.0186.e1.s1
PF3D7_1334200	chaperone binding protein	PF13.0190.e1.s1
PF3D7_1335100	merozoite surface protein 7 (MSP7)	PF13.0197
PF3D7_1335300	reticulocyte binding protein 2 homologue b (RH2b)	MAL13P1.176.e1.s1
PF3D7_1335300	reticulocyte binding protein 2 homologue b (RH2b)	MAL13P1.176.e1.s2
PF3D7_1335600	conserved Plasmodium protein	MAL13P1.178.e2
PF3D7_1335900	sporozoite surface protein 2 (TRAP)	PF13.0201
PF3D7_1337500	conserved Plasmodium protein	PF13.0210.e1.s1
PF3D7_1337500	conserved Plasmodium protein	PF13.0210.e1.s3
PF3D7_1338200	60S ribosomal protein L6-2	PF13.0213.e1.s1
PF3D7_1338300	elongation factor 1-gamma	PF13.0214.e2.s1
PF3D7_1339600	conserved Plasmodium protein	MAL13P1.201.e1.s1
PF3D7_1340500	conserved Plasmodium protein	PF13.0221.e1.s1
PF3D7_1340500	conserved Plasmodium protein	PF13.0221.e1.s2
PF3D7_1340600	RNA lariat debranching enzyme (DBR1)	PF13.0222.e1.s1
PF3D7_1341200	60S ribosomal protein L18	PF13.0224.e2.s1
PF3D7_1343600	UDP-N-acetylglucosamine pyrophosphorylase	MAL13P1.218.e2
PF3D7_1345700	isocitrate dehydrogenase (NADP), mitochondrial precursor (IDH)	PF13.0242.e1.s1
PF3D7_1346000	dynactin subunit 2	MAL13P1.230.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1346400	conserved Plasmodium protein	MAL13P1.234.e1.s1
PF3D7_1346400	conserved Plasmodium protein	MAL13P1.234.e1.s3
PF3D7_1346400	conserved Plasmodium protein	MAL13P1.234.e1.s4
PF3D7_1346400	conserved Plasmodium protein	MAL13P1.234.e1.s6
PF3D7_1346700	6-cysteine protein (P48/45)	PF13.0247.e1.s1
PF3D7_1346800	6-cysteine protein (P47)	PF13.0248.e1.s1
PF3D7_1347200	nucleoside transporter 1 (NT1)	PF13.0252.e1.s1
PF3D7_1347500	DNA/RNA-binding protein Alba 4 (ALBA4)	MAL13P1.237.e1
PF3D7_1347500	DNA/RNA-binding protein Alba 4 (ALBA4)	MAL13P1.237.e2
PF3D7_1349500	conserved Plasmodium protein	MAL13P1.249.e1.s4
PF3D7_1350100	lysine--tRNA ligase (KRS1)	PF13.0262.e2.s1
PF3D7_1350900	transcription factor with AP2 domain(s) (ApiAP2)	PF13.0267
PF3D7_1352500	thioredoxin-related protein	PF13.0272.e2
PF3D7_1353100	Plasmodium exported protein	PF13.0275.e2.s1
PF3D7_1353400	Ran-binding protein	PF13.0278
PF3D7_1353900	proteasome subunit	MAL13P1.270.e1.s1
PF3D7_1353900	proteasome subunit	MAL13P1.270.e2.s1
PF3D7_1354200	inositol-polyphosphate 5-phosphatase	PF13.0285
PF3D7_1354500	adenylosuccinate synthetase (adsS)	PF13.0287.e1.s1
PF3D7_1356800	serine/threonine protein kinase (ARK3)	MAL13P1.278.e1.s2
PF3D7_1356800	serine/threonine protein kinase (ARK3)	MAL13P1.278.e1.s3
PF3D7_1356800	serine/threonine protein kinase (ARK3)	MAL13P1.278.e1.s5
PF3D7_1357000	elongation factor 1-alpha	PF13.0304.e1.s1
PF3D7_1357100	elongation factor 1-alpha	PF13.0305
PF3D7_1357800	TCP-1/cpn60 chaperonin family	MAL13P1.283.e3.s1
PF3D7_1357900	pyrroline carboxylate reductase	MAL13P1.284.e5.s1
PF3D7_1358000	patatin-like phospholipase	MAL13P1.285.e2.s1
PF3D7_1360500	guanylyl cyclase beta (GCbeta)	PF13.0320.e13.s1
PF3D7_1360500	guanylyl cyclase beta (GCbeta)	PF13.0320.e13.s2
PF3D7_1360500	guanylyl cyclase beta (GCbeta)	PF13.0320.e9
PF3D7_1360700	SUMO ligase	MAL13P1.302.e3
PF3D7_1360900	polyadenylate-binding protein	MAL13P1.303.e2.s1
PF3D7_1361100	Sec24 subunit a (SEC24a)	PF13.0324.e1.s1
PF3D7_1361700	cytochrome c oxidase subunit 2	PF13.0327.e1
PF3D7_1361900	proliferating cell nuclear antigen (PCNA)	PF13.0328.e1.s1
PF3D7_1364200	conserved Plasmodium protein	PF13.0339.e1.s1
PF3D7_1364200	conserved Plasmodium protein	PF13.0339.e1.s2
PF3D7_1364400	conserved Plasmodium protein	MAL13P1.323.e1.s1
PF3D7_1364400	conserved Plasmodium protein	MAL13P1.323.e1.s2
PF3D7_1364400	conserved Plasmodium protein	MAL13P1.323.e1.s3
PF3D7_1364400	conserved Plasmodium protein	MAL13P1.323.e1.s4
PF3D7_1365500	glycine cleavage T protein (GCVT)	PF13.0345.e2.s1
PF3D7_1366300	conserved Plasmodium protein	MAL13P1.333.e2.s2
PF3D7_1366600	signal recognition particle receptor alpha subunit (SRPR-alpha)	PF13.0350
PF3D7_1366900	conserved Plasmodium protein	MAL13P1.336.e3.s1
PF3D7_1367100	U1 small nuclear ribonucleoprotein	MAL13P1.338.e1.s1
PF3D7_1368100	proteasome regulatory subunit	MAL13P1.343.e2.s1
PF3D7_1368200	RNAse L inhibitor protein	MAL13P1.344.e1.s1
PF3D7_1368800	DNA repair endonuclease	MAL13P1.346.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1368800	DNA repair endonuclease	MAL13P1.346.e1.s2
PF3D7_1401100	DnaJ protein	PF14.0013.e1.s1
PF3D7_1401100	DnaJ protein	PF14.0013.e2.s1
PF3D7_1401600	Plasmodium exported protein (PHISTb)	PF14.0018.e2.s1
PF3D7_1401600	Plasmodium exported protein (PHISTb)	PF14.0018.e3.s1
PF3D7_1403200; PF3D7_1403300	conserved Plasmodium protein	PF14.0031.e1.s2
PF3D7_1403200; PF3D7_1403300	conserved Plasmodium protein	PF14.0031.e2.s1
PF3D7_1403200; PF3D7_1403300	conserved Plasmodium protein	PF14.0031.e4.s2
PF3D7_1403800	nuclear formin-like protein (MISFIT)	PF14.0035.e1.s2
PF3D7_1403900	phosphatase	PF14.0036.e1.s1
PF3D7_1404900	conserved Plasmodium protein	PF14.0046.e1.s1
PF3D7_1405400	DNA mismatch repair protein	PF14.0051.e1.s1
PF3D7_1405400	DNA mismatch repair protein	PF14.0051.e2.s1
PF3D7_1405400	DNA mismatch repair protein	PF14.0051.e4.s1
PF3D7_1406200	conserved Plasmodium protein	PF14.0059.e1.s1
PF3D7_1406200	conserved Plasmodium protein	PF14.0059.e1.s3
PF3D7_1407000	LCCL domain-containing protein (CCp3)	PF14.0067.e1.s2
PF3D7_1407700	conserved Plasmodium protein	PF14.0074.e1
PF3D7_1407800	plasmepsin IV (PM4)	PF14.0075.e1.s1
PF3D7_1407900	plasmepsin I (PMI)	PF14.0076.e1.s1
PF3D7_1408000	plasmepsin II	PF14.0077.e1.s1
PF3D7_1408400	DNA-repair helicase	PF14.0081.e1.s1
PF3D7_1408400	DNA-repair helicase	PF14.0081.e1.s2
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e1.s2
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e1.s3
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e1.s4
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e2.s1
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e2.s2
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e2.s3
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e3.s1
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e3.s2
PF3D7_1408700	conserved Plasmodium protein	PF14.0084.e4.s1
PF3D7_1408800	conserved Plasmodium protein	PF14.0085.e1.s1
PF3D7_1409800	RNA binding protein Bruno (HoBo)	PF14.0096.e1.s1
PF3D7_1410400	rhoptry-associated protein 1 (RAP1)	PF14.0102.e1.s1
PF3D7_1410600	eukaryotic translation initiation factor 2 gamma subunit	PF14.0104
PF3D7_1412500	actin II (ACT2)	PF14.0124.e1.s1
PF3D7_1412500	actin II (ACT2)	PF14.0124.e2.s1
PF3D7_1414600	RNA guanylyltransferase (Pgt1)	PF14.0144.e1.s1
PF3D7_1414700	ubiquitin carboxyl-terminal hydrolase	PF14.0145.s1
PF3D7_1414700	ubiquitin carboxyl-terminal hydrolase	PF14.0145.s2
PF3D7_1415300	RNA-binding protein Nova-1	PF14.0151.e1.s1
PF3D7_1416500	NADP-specific glutamate dehydrogenase (GDH1)	PF14.0164.e1.s1
PF3D7_1416600	conserved Plasmodium protein	PF14.0165.e1.s2
PF3D7_1416600	conserved Plasmodium protein	PF14.0165.e1.s4
PF3D7_1417200	NOT family protein	PF14.0170.e1.s1
PF3D7_1417200	NOT family protein	PF14.0170.e1.s3

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1417200	NOT family protein	PF14.0170.e1.s4
PF3D7_1417200	NOT family protein	PF14.0170.e1.s5
PF3D7_1417500	pseudouridine synthase	PF14.0174.e1.s1
PF3D7_1417800	DNA replication licensing factor MCM2 (MCM2)	PF14.0177.e2.s1
PF3D7_1418100	liver specific protein 1 (LISP1)	PF14.0179.s3
PF3D7_1418100	liver specific protein 1 (LISP1)	PF14.0179.s4
PF3D7_1419200	conserved Plasmodium protein	PF14.0186.e1.s1
PF3D7_1419300	glutathione S-transferase (GST)	PF14.0187.e2.s1
PF3D7_1419400	conserved Plasmodium membrane protein	PF14.0188.e1.s1
PF3D7_1419400	conserved Plasmodium membrane protein	PF14.0188.e1.s2
PF3D7_1419800.1; PF3D7_1419800.2	glutathione reductase (GR)	PF14.0192.e3.s1
PF3D7_1420200	tetratricopeptide repeat family protein	PF14.0196
PF3D7_1420400	glycine--tRNA ligase (GlyRS)	PF14.0198.e1.s1
PF3D7_1420700	surface protein, Pf113 (Pf113)	PF14.0201.e2
PF3D7_1421200	40S ribosomal protein S25	PF14.0205.e1.s1
PF3D7_1422700	conserved Plasmodium protein	PF14.0217.e1.s1
PF3D7_1422700	conserved Plasmodium protein	PF14.0217.e1.s2
PF3D7_1423700	conserved Plasmodium protein	PF14.0228.e1.s1
PF3D7_1423700	conserved Plasmodium protein	PF14.0228.e1.s2
PF3D7_1424100	60S ribosomal protein L5	PF14.0230.e2
PF3D7_1424400	60S ribosomal protein L7-3	PF14.0231.e3
PF3D7_1425600	zinc finger protein	PF14.0236.s2
PF3D7_1426100	basic transcription factor 3b	PF14.0241
PF3D7_1426500	ATP-binding cassette sub-family G member 2 (ABCG2)	PF14.0244.e1.s1
PF3D7_1426700	phosphoenolpyruvate carboxylase (PEPC)	PF14.0246.e1.s2
PF3D7_1427000	conserved Plasmodium protein	PF14.0249
PF3D7_1427400.1; PF3D7_1427400.2	conserved Plasmodium membrane protein	PF14.0253.e2
PF3D7_1427900	conserved protein	PF14.0257
PF3D7_1428300	proliferation-associated protein 2g4	PF14.0261.e2
PF3D7_1430700	NADP-specific glutamate dehydrogenase (GDH2)	PF14.0286.e3
PF3D7_1431100	conserved Plasmodium protein	PF14.0290.e1.s1
PF3D7_1431300	large ribosomal subunit associated GTPase	PF14.0292.e1.s1
PF3D7_1432900	SF-assemblin	PF14.0311.e1.s1
PF3D7_1433400	conserved Plasmodium membrane protein	PF14.0315.e1
PF3D7_1433400	conserved Plasmodium membrane protein	PF14.0315.e2.s2
PF3D7_1433400	conserved Plasmodium membrane protein	PF14.0315.e2.s3
PF3D7_1433400	conserved Plasmodium membrane protein	PF14.0315.e2.s5
PF3D7_1433500	DNA topoisomerase II	PF14.0316.e1.s1
PF3D7_1433500	DNA topoisomerase II	PF14.0316.e1.s2
PF3D7_1434500	dynein-related AAA-type ATPase	PF14.0326.e2.s1
PF3D7_1434500	dynein-related AAA-type ATPase	PF14.0326.e2.s2
PF3D7_1434500	dynein-related AAA-type ATPase	PF14.0326.e2.s3
PF3D7_1434500	dynein-related AAA-type ATPase	PF14.0326.e2.s4
PF3D7_1434500	dynein-related AAA-type ATPase	PF14.0326.e2.s5
PF3D7_1434600	methionine aminopeptidase 2 (MetAP2)	PF14.0327.e1.s1
PF3D7_1435300	NAD(P)H-dependent glutamate synthase	PF14.0334.e1.s1
PF3D7_1435300	NAD(P)H-dependent glutamate synthase	PF14.0334.e1.s2
PF3D7_1435300	NAD(P)H-dependent glutamate synthase	PF14.0334.e1.s4

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1435700.1; PF3D7_1435700.2	ataxin-2 like protein	PF14.0338.e1.s1
PF3D7_1435700.1; PF3D7_1435700.2	ataxin-2 like protein	PF14.0338.e9.s1
PF3D7_1436300	translocon component PTEX150 (PTEX150)	PF14.0344.e1.s1
PF3D7_1436400; PF3D7_1436500	conserved Plasmodium protein; GTP-binding protein	PF14.0345.e1.s1
PF3D7_1436400; PF3D7_1436500	conserved Plasmodium protein; GTP-binding protein	PF14.0345.e1.s2
PF3D7_1436400; PF3D7_1436500	conserved Plasmodium protein; GTP-binding protein	PF14.0345.e2
PF3D7_1437200	ribonucleoside-diphosphate reductase, large subunit	PF14.0352.e1
PF3D7_1437900	HSP40, subfamily A	PF14.0359.e2.s1
PF3D7_1438000	eukaryotic translation initiation factor eIF2A	PF14.0360.e1.s1
PF3D7_1438100	secretory complex protein 62 (SEC62)	PF14.0361
PF3D7_1439100	DEAD/DEAH box helicase	PF14.0370.e1.s1
PF3D7_1439100	DEAD/DEAH box helicase	PF14.0370.e1.s2
PF3D7_1439100	DEAD/DEAH box helicase	PF14.0370.e2.s1
PF3D7_1439100	DEAD/DEAH box helicase	PF14.0370.e2.s2
PF3D7_1439300	conserved Plasmodium protein	PF14.0372.e2.s2
PF3D7_1439400	ubiquinol-cytochrome c reductase iron-sulfur subunit	PF14.0373.e1.s1
PF3D7_1439800	vesicle-associated membrane protein	PF14.0377.e1
PF3D7_1439900	triosephosphate isomerase (TIM)	PF14.0378.e2.s1
PF3D7_1440500	allantoicase	PF14.0384.e1.s1
PF3D7_1441200	60S ribosomal protein L1	PF14.0391.e2
PF3D7_1441300	serine/threonine protein kinase	PF14.0392.e4.s1
PF3D7_1441300	serine/threonine protein kinase	PF14.0392.e5.s1
PF3D7_1441400	FACT complex subunit SSRP1 (FACT-S)	PF14.0393.e1.s1
PF3D7_1442300	tRNA binding protein	PF14.0401.e2.s1
PF3D7_1442400	conserved Plasmodium protein	PF14.0402.e1.s1
PF3D7_1442400	conserved Plasmodium protein	PF14.0402.e1.s3
PF3D7_1442700	conserved Plasmodium protein	PF14.0405.e2.s1
PF3D7_1442700	conserved Plasmodium protein	PF14.0405.e2.s2
PF3D7_1442700	conserved Plasmodium protein	PF14.0405.e2.s3
PF3D7_1442900	guanine nucleotide exchange factor (SEC7)	PF14.0407.s2
PF3D7_1443400	conserved Plasmodium protein	PF14.0412.e2
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e1.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e10.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e11.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e3.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e6.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e7.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e8.s2
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e9.s1
PF3D7_1444100	conserved Plasmodium protein	PF14.0419.e9.s2
PF3D7_1444300	apicoplast 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT)	PF14.0421.e2.s1
PF3D7_1444800	fructose-bisphosphate aldolase (FBPA)	PF14.0425.e2.s1
PF3D7_1445300	mitochondrial ribosomal protein S29 precursor	PF14.0430.e1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1445600	RNA binding protein	PF14.0433.e4
PF3D7_1445700	conserved Plasmodium protein	PF14.0434.e3.s1
PF3D7_1445900	DEAD/DEAH box ATP-dependent RNA helicase	PF14.0436.e1.s1
PF3D7_1445900	DEAD/DEAH box ATP-dependent RNA helicase	PF14.0437.e2.s1
PF3D7_1446800	heme detoxification protein (HDP)	PF14.0446.e1.s1
PF3D7_1446800	heme detoxification protein (HDP)	PF14.0446.e3.s1
PF3D7_1447800	conserved Plasmodium protein	PF14.0454.e1.s1
PF3D7_1447800	conserved Plasmodium protein	PF14.0454.e1.s2
PF3D7_1448000	U3 snoRNA-associated small subunit rRNA processing protein	PF14.0456.e2.s1
PF3D7_1448000	U3 snoRNA-associated small subunit rRNA processing protein	PF14.0456.e2.s2
PF3D7_1448300	conserved Plasmodium protein	PF14.0461.s1
PF3D7_1448300	conserved Plasmodium protein	PF14.0461.s2
PF3D7_1448500	conserved Plasmodium protein	PF14.0463.e1.s1
PF3D7_1448500	conserved Plasmodium protein	PF14.0463.e1.s3
PF3D7_1448500	conserved Plasmodium protein	PF14.0463.e1.s4
PF3D7_1449400	DNA replication related protein	PF14.0470.e2.s1
PF3D7_1449400	DNA replication related protein	PF14.0470.e2.s2
PF3D7_1450500	conserved Plasmodium protein	PF14.0480.e3.s1
PF3D7_1450500	conserved Plasmodium protein	PF14.0480.e3.s2
PF3D7_1451800	sortilin	PF14.0493.e1
PF3D7_1451900	small subunit rRNA processing factor	PF14.0494.e1.s2
PF3D7_1452000	rhoptry neck protein 2 (RON2)	PF14.0495.s1
PF3D7_1452000	rhoptry neck protein 2 (RON2)	PF14.0495.s2
PF3D7_1452000	rhoptry neck protein 2 (RON2)	PF14.0495.s3
PF3D7_1452900; PF3D7_1453000	conserved Plasmodium protein	PF14.0504.e2.s1
PF3D7_1452900; PF3D7_1453000	conserved Plasmodium protein	PF14.0504.e3.s1
PF3D7_1453700	co-chaperone p23 (P23)	PF14.0510.e2.s1
PF3D7_1453800	glucose-6-phosphate dehydrogenase-6-phosphogluconolactonase (G6PDH)	PF14.0511.e1.s1
PF3D7_1454200	conserved Plasmodium protein	PF14.0515.s1
PF3D7_1454200	conserved Plasmodium protein	PF14.0515.s2
PF3D7_1454700	6-phosphogluconate dehydrogenase, decarboxylating	PF14.0520.e1.s1
PF3D7_1455800	LCCL domain-containing protein (CCp2)	PF14.0532.e1.s1
PF3D7_1456500	conserved Plasmodium protein	PF14.0538.s1
PF3D7_1456500	conserved Plasmodium protein	PF14.0538.s2
PF3D7_1456500	conserved Plasmodium protein	PF14.0538.s3
PF3D7_1457300	conserved Plasmodium protein	PF14.0546.e1.s1
PF3D7_1457700	large ribosomal subunit nuclear export factor	PF14.0550.e1.s1
PF3D7_1457900	conserved Plasmodium protein	PF14.0552.e1.s2
PF3D7_1457900	conserved Plasmodium protein	PF14.0552.e1.s4
PF3D7_1460700	60S ribosomal protein L27	PF14.0579.e2.s1
PF3D7_1461800	conserved Plasmodium protein	PF14.0588.e1.s1
PF3D7_1461900	valine--tRNA ligase	PF14.0589.e1.s1
PF3D7_1461900	valine--tRNA ligase	PF14.0589.e1.s2
PF3D7_1462300	conserved Plasmodium protein	PF14.0593.e1.s1
PF3D7_1462300	conserved Plasmodium protein	PF14.0593.e1.s2

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1462400	conserved Plasmodium protein	PF14.0594.e1.s2
PF3D7_1462400	conserved Plasmodium protein	PF14.0594.e1.s3
PF3D7_1462700	cytochrome c1 precursor	PF14.0597.e2.s1
PF3D7_1463200	replication factor C3 (RFC3)	PF14.0601.e2.s1
PF3D7_1463300	DNA polymerase alpha subunit	PF14.0602.e1.s1
PF3D7_1464000	YL1 nuclear protein	PF14.0608.e1.s1
PF3D7_1464700	ATP synthase (C/AC39) subunit	PF14.0615.e1.s1
PF3D7_1465300	tRNA 3'-trailer sequence RNase	PF14.0620.e1
PF3D7_1465800	dynein beta chain	PF14.0626.e4.s1
PF3D7_1465800	dynein beta chain	PF14.0626.e4.s2
PF3D7_1465800	dynein beta chain	PF14.0626.e5.s1
PF3D7_1465800	dynein beta chain	PF14.0626.e5.s2
PF3D7_1465800	dynein beta chain	PF14.0626.e5.s4
PF3D7_1465800	dynein beta chain	PF14.0626.e6.s1
PF3D7_1465900	40S ribosomal protein S3	PF14.0627.e3.s1
PF3D7_1466200	conserved Plasmodium protein	PF14.0631.e1.s1
PF3D7_1466200	conserved Plasmodium protein	PF14.0631.e1.s2
PF3D7_1466200	conserved Plasmodium protein	PF14.0631.e1.s3
PF3D7_1466300	26S proteasome regulatory subunit	PF14.0632.e1.s1
PF3D7_1466300	26S proteasome regulatory subunit	PF14.0632.e1.s2
PF3D7_1466400	transcription factor with AP2 domain(s) (ApiAP2)	PF14.0633.e1.s1
PF3D7_1467900	rab GTPase activator	PF14.0647.e3.s2
PF3D7_1468000	conserved Plasmodium protein	PF14.0648.e10
PF3D7_1468000	conserved Plasmodium protein	PF14.0648.e11
PF3D7_1468000	conserved Plasmodium protein	PF14.0648.e3
PF3D7_1468000	conserved Plasmodium protein	PF14.0648.e8.s2
PF3D7_1468100	conserved Plasmodium protein	PF14.0649.e2.s1
PF3D7_1468100	conserved Plasmodium protein	PF14.0649.e2.s2
PF3D7_1468700	eukaryotic initiation factor 4A (eIF4A)	PF14.0655.e1.s1
PF3D7_1468700	eukaryotic initiation factor 4A (eIF4A)	PF14.0655.e2.s1
PF3D7_1469600	biotin carboxylase subunit of acetyl CoA carboxylase (ACC1)	PF14.0664.e1.s1
PF3D7_1469600	biotin carboxylase subunit of acetyl CoA carboxylase (ACC1)	PF14.0664.e1.s2
PF3D7_1469600	biotin carboxylase subunit of acetyl CoA carboxylase (ACC1)	PF14.0664.e1.s3
PF3D7_1469600	biotin carboxylase subunit of acetyl CoA carboxylase (ACC1)	PF14.0664.e1.s4
PF3D7_1470100	conserved Plasmodium protein	PF14.0668.e1.s1
PF3D7_1470100	conserved Plasmodium protein	PF14.0668.e1.s2
PF3D7_1470100	conserved Plasmodium protein	PF14.0668.e1.s3
PF3D7_1471100	exported protein 2 (EXP2)	PF14.0678.e2.s1
PF3D7_1471100	exported protein 2 (EXP2)	PF14.0678.e3.s1
PF3D7_1471400	diacylglycerol kinase	PF14.0681.e2.s1
PF3D7_1472200	histone deacetylase	PF14.0690.e1.s1
PF3D7_1472200	histone deacetylase	PF14.0690.e1.s2
PF3D7_1472200	histone deacetylase	PF14.0690.e1.s3
PF3D7_1473100	GTPase activator	PF14.0699.e1.s1
PF3D7_1473100	GTPase activator	PF14.0699.e2.s1
PF3D7_1473200	DnaJ protein	PF14.0700.e1.s1

Appendix A. 1494 *P. falciparum* antigens on the Malian protein microarray (Chapter 1) meeting the inclusion criteria (continued).

Gene ID	Description	Spot ID
PF3D7_1474800	proteosome subunit alpha type 1	PF14.0716.e3.s1
PF3D7_1475400	cysteine repeat modular protein 4 (CRMP4)	PF14.0722.e2.s1
PF3D7_1475400	cysteine repeat modular protein 4 (CRMP4)	PF14.0722.e2.s2
PF3D7_1475400	cysteine repeat modular protein 4 (CRMP4)	PF14.0722.e3.s2
PF3D7_1475400	cysteine repeat modular protein 4 (CRMP4)	PF14.0722.e3.s3
PF3D7_1475400	cysteine repeat modular protein 4 (CRMP4)	PF14.0722.e3.s4
PF3D7_1475500	LCCL domain-containing protein (CCp1)	PF14.0723.e1.s1
PF3D7_1475500	LCCL domain-containing protein (CCp1)	PF14.0723.e1.s2
PF3D7_1475800	conserved Plasmodium protein	PF14.0726.e1
PF3D7_1476600	Plasmodium exported protein	PF14.0736.e2
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	PF14.0751.e1
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	PF14.0751.e2
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	PF14.0751.e3

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria.

Gene ID	Description	Exon & Segment
PF3D7_0102200	ring-infected erythrocyte surface antigen (RESA)	exon 2 segment 1
PF3D7_0102200	ring-infected erythrocyte surface antigen (RESA)	exon 2 segment 2
PF3D7_0102500	erythrocyte binding antigen-181 (EBA181)	exon 1 segment 2
PF3D7_0102800	conserved Plasmodium protein	
PF3D7_0102800	conserved Plasmodium protein	
PF3D7_0104000	thrombospondin-related sporozoite protein (TRSP)	exon 3 segment 1
PF3D7_0104600	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0104800	novel putative transporter 1 (NPT1)	
PF3D7_0104800	novel putative transporter 1 (NPT1)	exon 1 segment 1
PF3D7_0107600	serine/threonine protein kinase, putative	exon 1 segment 1
PF3D7_0107600	serine/threonine protein kinase, putative	exon 1 segment 2
PF3D7_0107600	serine/threonine protein kinase, putative	exon 2
PF3D7_0108300	conserved Plasmodium protein	segment 2
PF3D7_0108300	conserved Plasmodium protein	segment 3
PF3D7_0110500	bromodomain protein, putative	exon 1 segment 2
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3) (SURF1.3)	exon 1 segment 1
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3) (SURF1.3)	exon 2 segment 1
PF3D7_0115000	surface-associated interspersed protein 1.3 (SURFIN 1.3) (SURF1.3)	exon 3 segment 2
PF3D7_0200100	erythrocyte membrane protein 1, PfEMP1 (VAR)	
PF3D7_0201500	Plasmodium exported protein (hyp9)	
PF3D7_0202400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0203100	protein kinase, putative; conserved Plasmodium protein	exon 2 segment 2
PF3D7_0205900	proteasome 26S regulatory subunit, putative	exon 2
PF3D7_0206500	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0206500	conserved Plasmodium protein	exon 1 segment 2
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206800	merozoite surface protein 2 (MSP2)	
PF3D7_0206900.1	merozoite surface protein 5 (MSP5)	
PF3D7_0206900.1	merozoite surface protein 5 (MSP5)	exon 1
PF3D7_0207000	merozoite surface protein 4 (MSP4)	
PF3D7_0207000	merozoite surface protein 4 (MSP4)	exon 1
PF3D7_0207000	merozoite surface protein 4 (MSP4)	exon 1
PF3D7_0207100	conserved Plasmodium protein	segment 1
PF3D7_0207300	serine repeat antigen 8 (SERA8)	exon 3 segment 1
PF3D7_0207300	serine repeat antigen 8 (SERA8)	exon 5 segment 1
PF3D7_0207400	serine repeat antigen 7 (SERA7)	
PF3D7_0207600	serine repeat antigen 5 (SERA5)	exon 2 segment 1
PF3D7_0207600	serine repeat antigen 5 (SERA5)	exon 4 segment 1
PF3D7_0207700	serine repeat antigen 4 (SERA4)	
PF3D7_0208000	serine repeat antigen 1 (SERA1)	
PF3D7_0208000	serine repeat antigen 1 (SERA1)	
PF3D7_0209000	6-cysteine protein, Gametocyte surface protein (P230)	

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0209000	6-cysteine protein (P230)	segment 1
PF3D7_0209000	6-cysteine protein (P230)	segment 3
PF3D7_0209000	6-cysteine protein (P230)	segment 4
PF3D7_0214100	protein transport protein sec31 (SEC31)	exon 1 segment 1
PF3D7_0215200	conserved Plasmodium protein	
PF3D7_0217000	conserved Plasmodium membrane protein	
PF3D7_0220000	liver stage antigen 3 (LSA3)	exon 2 segment 1
PF3D7_0220000	liver stage antigen 3 (LSA3)	exon 2 segment 2
PF3D7_0220100	DnaJ protein, putative	exon 2 segment 1
PF3D7_0223300	erythrocyte membrane protein 1, PfEMP1 (VAR)	
PF3D7_0301000	acyl-CoA synthetase (ACS2)	
PF3D7_0302800	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0304000	inner membrane complex protein 1a, putative (IMC1a)	exon 4 segment 1
PF3D7_0304600	circumsporozoite (CS) protein (CSP)	
PF3D7_0304600	circumsporozoite (CS) protein (CSP)	exon 1 segment 1
PF3D7_0306900	40S ribosomal protein S23, putative	exon 3 segment 1
PF3D7_0310300	phosphoglycerate mutase, putative	exon 1 segment 2
PF3D7_0311900	conserved Plasmodium protein	
PF3D7_0313500	GTP-binding protein EngA, putative	
PF3D7_0314900	conserved Plasmodium membrane protein	
PF3D7_0315900	conserved Plasmodium protein	
PF3D7_0315900	conserved Plasmodium protein	
PF3D7_0319700	ABC transporter, putative	exon 1 segment 1
PF3D7_0401700	Plasmodium exported protein, pseudogene	
PF3D7_0401800	Plasmodium exported protein (PHISTb) (PfD80)	exon 2 segment 1
PF3D7_0401900	acyl-CoA synthetase (ACS6)	
PF3D7_0401900	acyl-CoA synthetase (ACS6)	exon 1 segment 1
PF3D7_0402200	surface-associated interspersed protein 4.1 (SURFIN 4.1), pseudogene (SURF4.1)	
PF3D7_0402400	Plasmodium exported protein (GEXP18)	
PF3D7_0403500	ubiquitin specific protease, putative	
PF3D7_0404500	6-cysteine protein (P52)	
PF3D7_0404600	conserved Plasmodium membrane protein	segment 5
PF3D7_0405300	sequestrin (LISP2)	exon 1 segment 1
PF3D7_0405300	sequestrin (LISP2)	exon 1 segment 2
PF3D7_0405400	pre-mRNA-processing-splicing factor 8, putative (PRPF8)	exon 2 segment 1
PF3D7_0406200	sexual stage-specific protein precursor (Pfs16)	
PF3D7_0407800	conserved Plasmodium protein	exon 1 segment 2
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 1 segment 1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 3 segment 1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 4 segment 1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 5 segment 1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 6 segment 1
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 7 segment 1

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0408700	perforin like protein 1,sporozoite micronemal protein essential for cell traversal (SPECT2)	exon 8 segment 1
PF3D7_0408900.1	peptidase, M22 family, putative	exon 1 segment 1
PF3D7_0408900.1	peptidase, M22 family, putative	exon 2 segment 1
PF3D7_0408900.1	peptidase, M22 family, putative	exon 3 segment 1
PF3D7_0414700	GTP binding protein, putative	
PF3D7_0418700	RNA-binding protein NOB1, putative	
PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0420700	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0422100	transmembrane emp24 domain-containing protein, putative	
PF3D7_0423700	early transcribed membrane protein 4 (ETRAMP4)	
PF3D7_0424100	reticulocyte binding protein homologue 5 (RH5)	
PF3D7_0424800	Plasmodium exported protein (PHISTb)	
PF3D7_0500800	mature parasite-infected erythrocyte surface antigen,erythrocyte membrane protein 2 (MESA)	exon 2 segment 1
PF3D7_0501100.1	heat shock protein 40, type II (HSP40)	exon 3 segment 1
PF3D7_0501100.1	heat shock protein 40, type II (HSP40)	exon 4 segment 1
PF3D7_0501200	parasite-infected erythrocyte surface protein (PIESP2)	exon 2
PF3D7_0501800	chromosome assembly factor 1 (CAF1)	exon 1 segment 1
PF3D7_0501800	chromosome assembly factor 1 (CAF1)	exon 1 segment 1
PF3D7_0501800	chromosome assembly factor 1 (CAF1)	exon 1 segment 2
PF3D7_0502200	conserved Plasmodium membrane protein	exon 2 segment 1
PF3D7_0503600	myosin B (MyoB)	exon 1 segment 1
PF3D7_0503600	myosin B (MyoB)	exon 5 segment 1
PF3D7_0503600	myosin B (MyoB)	exon 6 segment 1
PF3D7_0503600	myosin B (MyoB)	exon 7 segment 1
PF3D7_0503600	myosin B (MyoB)	exon 8 segment 1
PF3D7_0505300	UDP-N-acetyl glucosamine:UMP antiporter	
PF3D7_0506200	transcription initiation factor TFiid, TATA-binding protein (TBP)	
PF3D7_0509000	SNAP protein (soluble N-ethylmaleimide-sensitive factor Attachment Protein), putative	exon 1 segment 1
PF3D7_0509400	RNA polymerase I (RNAPI)	exon 1 segment 1
PF3D7_0509400	RNA polymerase I (RNAPI)	exon 1 segment 2
PF3D7_0509400	RNA polymerase I (RNAPI)	exon 1 segment 3
PF3D7_0509700	conserved Plasmodium protein	exon 2 segment 1
PF3D7_0509700	conserved Plasmodium protein	exon 4 segment 1
PF3D7_0509700	conserved Plasmodium protein	exon 6 segment 1
PF3D7_0511200	stearoyl-CoA desaturase (SCD)	
PF3D7_0511200	stearoyl-CoA desaturase (SCD)	exon 1
PF3D7_0511200	stearoyl-CoA desaturase (SCD)	exon 2
PF3D7_0511400	conserved Plasmodium protein	
PF3D7_0511400	conserved Plasmodium protein	
PF3D7_0513200	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0513200	conserved Plasmodium protein	exon 2 segment 1
PF3D7_0513200	conserved Plasmodium protein	exon 2 segment 2
PF3D7_0513200	conserved Plasmodium protein	exon 2 segment 3
PF3D7_0515100	rhomboid protease ROM9 (ROM9)	exon 1 segment 1
PF3D7_0515300	phosphatidylinositol 3-kinase (PI3K)	segment 1
PF3D7_0515300	phosphatidylinositol 3-kinase (PI3K)	segment 2
PF3D7_0515700	glideosome-associated protein 40, putative (GAP40)	

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0516100	cation-transporting ATPase 1 (ATPase1)	exon 2 segment 1
PF3D7_0516300	tRNA pseudouridine synthase, putative	
PF3D7_0516300	tRNA pseudouridine synthase, putative	
PF3D7_0519100	mitochondrial ribosomal protein L14 precursor, putative	exon 1 segment 1
PF3D7_0522400	conserved Plasmodium protein	exon 3 segment 3
PF3D7_0522400	conserved Plasmodium protein	exon 4 segment 2
PF3D7_0525100	acyl-CoA synthetase (ACS10)	exon 1 segment 1
PF3D7_0526100	conserved Plasmodium membrane protein	
PF3D7_0526100	conserved Plasmodium membrane protein	
PF3D7_0527600	conserved Plasmodium protein	
PF3D7_0529400.1	conserved Plasmodium protein	exon 5 segment 1
PF3D7_0529800	conserved Plasmodium protein	
PF3D7_0532100	early transcribed membrane protein 5 (ETRAMP5)	
PF3D7_0532300	Plasmodium exported protein (PHISTb)	exon 2 segment 1
PF3D7_0532400	Plasmodium exported protein (PHISTb)	exon 2 segment 1
PF3D7_0607300	uroporphyrinogen III decarboxylase (UROD)	
PF3D7_0607900	conserved Plasmodium protein	
PF3D7_0608900	conserved Plasmodium protein	
PF3D7_0615800	conserved Plasmodium protein	
PF3D7_0616000	pyridoxine kinase (PdxK)	
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	segment 3
PF3D7_0617400	erythrocyte membrane protein 1, PfEMP1 (VAR)	segment 4
PF3D7_0619000.1	conserved Plasmodium protein	segment 1
PF3D7_0619000.1	conserved Plasmodium protein	segment 2
PF3D7_0620000	conserved Plasmodium protein	
PF3D7_0620400	merozoite surface protein 10 (MSP10)	
PF3D7_0624800	conserved Plasmodium protein	
PF3D7_0625600	poly(A) polymerase PAP, putative	
PF3D7_0626900	mitochondrial ribosomal protein L46 precursor, putative	
PF3D7_0628200	protein kinase PK4 (PK4)	segment 2
PF3D7_0630600	conserved Plasmodium protein	
PF3D7_0702300	sporozoite threonine and asparagine-rich protein (STARP)	
PF3D7_0702300	sporozoite threonine and asparagine-rich protein (STARP)	exon 2
PF3D7_0702400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0703700	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0704300	conserved Plasmodium membrane protein	segment 1
PF3D7_0705000	methyltransferase, putative	exon 1 segment 1
PF3D7_0705000	methyltransferase, putative	exon 1 segment 2
PF3D7_0707700	ubiquitin-protein ligase e3, putative	
PF3D7_0707800	RAP protein, putative	exon 1 segment 1
PF3D7_0707800	RAP protein, putative	exon 1 segment 2
PF3D7_0709100	Cg1 protein	exon 1 segment 1
PF3D7_0710000	conserved Plasmodium protein	exon 7 segment 1
PF3D7_0710400	nucleotide excision repair protein, putative	
PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0711700	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0716200	conserved Plasmodium protein	
PF3D7_0716300	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0718300	cysteine repeat modular protein 2 (CRMP2)	exon 3 segment 1

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0724700	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0724700	conserved Plasmodium protein	exon 2 segment 1
PF3D7_0724700	conserved Plasmodium protein	exon 2 segment 2
PF3D7_0724800	kelch protein, putative	exon 1
PF3D7_0725100	conserved Plasmodium membrane protein	exon 1 segment 1
PF3D7_0727900	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0727900	conserved Plasmodium protein	exon 1 segment 2
PF3D7_0728100	conserved Plasmodium membrane protein	segment 1
PF3D7_0728100	conserved Plasmodium membrane protein	segment 2
PF3D7_0728100	conserved Plasmodium membrane protein	segment 3
PF3D7_0728100	conserved Plasmodium membrane protein	segment 4
PF3D7_0728100	conserved Plasmodium membrane protein	segment 5
PF3D7_0728100	conserved Plasmodium membrane protein	segment 6
PF3D7_0730300	transcription factor with AP2 domain(s) (AP2-L)	exon 1 segment 2
PF3D7_0730500	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0730500	conserved Plasmodium protein	exon 1 segment 2
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	segment 1
PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	segment 2
PF3D7_0731600	acyl-CoA synthetase (ACS5)	exon 1 segment 1
PF3D7_0731600	acyl-CoA synthetase (ACS5)	exon 1 segment 1
PF3D7_0800200	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 1
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 2
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0800300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0800700	surface-associated interspersed protein 8.3 (SURFIN 8.3) (SURF8.3)	exon 1 segment 1
PF3D7_0800700	surface-associated interspersed protein 8.3 (SURFIN 8.3) (SURF8.3)	exon 2 segment 1
PF3D7_0800700	surface-associated interspersed protein 8.3 (SURFIN 8.3) (SURF8.3)	exon 3 segment 2
PF3D7_0801000	Plasmodium exported protein (PHISTc)	exon 2 segment 1
PF3D7_0801000	Plasmodium exported protein (PHISTc)	exon 2 segment 2
PF3D7_0801700	sentrin-specific protease 2, putative (SENP2)	
PF3D7_0802500	inositol phosphatase, putative	exon 1 segment 1
PF3D7_0802500	inositol phosphatase, putative	exon 2 segment 1
PF3D7_0804400	methionine aminopeptidase 1c, putative (MetAP1c)	
PF3D7_0804400	methionine aminopeptidase 1c, putative (MetAP1c)	
PF3D7_0804500	conserved Plasmodium membrane protein	exon 1 segment 4
PF3D7_0806600	kinesin-like protein, putative	exon 2 segment 1
PF3D7_0806600	kinesin-like protein, putative	exon 3 segment 1
PF3D7_0806600	kinesin-like protein, putative	exon 5 segment 1
PF3D7_0806800	vacuolar proton translocating ATPase subunit A, putative	exon 1 segment 2
PF3D7_0807700	serine protease, putative	
PF3D7_0807700	serine protease, putative	
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 1
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 2
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0808600	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_0809200	asparagine-rich antigen Pfa55-14 (pfa55-14)	exon 1 segment 1
PF3D7_0809200	asparagine-rich antigen Pfa55-14 (pfa55-14)	exon 1 segment 2
PF3D7_0809400	conserved Plasmodium protein	
PF3D7_0812000	conserved Plasmodium protein	
PF3D7_0814000	60S ribosomal protein L13-2, putative	exon 1 segment 1
PF3D7_0816500	small heat shock protein HSP20, putative (HSP20)	exon 2 segment 1
PF3D7_0817900	high mobility group protein B2 (HMGB2)	exon 1 segment 1
PF3D7_0818200	14-3-3 protein (14-3-3I)	exon 3
PF3D7_0818900	heat shock protein 70 (HSP70)	
PF3D7_0819800	conserved Plasmodium protein	exon 15 segment 1
PF3D7_0820300	conserved Plasmodium protein	
PF3D7_0820800	conserved Plasmodium protein	
PF3D7_0822600	protein transport protein Sec23 (SEC23)	exon 1 segment 1
PF3D7_0822700	conserved Plasmodium protein	
PF3D7_0822700	conserved Plasmodium protein	exon 1
PF3D7_0822700	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0822700	conserved Plasmodium protein	exon 3 segment 1
PF3D7_0826500	ubiquitin conjugation factor E4 B, putative (UBE4B)	exon 1 segment 2
PF3D7_0827100	translation initiation factor IF-2, putative	segment 1
PF3D7_0827100	translation initiation factor IF-2, putative	segment 2
PF3D7_0827600	conserved Plasmodium protein	
PF3D7_0828800	GPI-anchored micronemal antigen (GAMA)	
PF3D7_0829500	conserved Plasmodium protein	exon 1
PF3D7_0829500	conserved Plasmodium protein	exon 2
PF3D7_0829500	conserved Plasmodium protein	exon 2 segment 1
PF3D7_0829500	conserved Plasmodium protein	exon 3
PF3D7_0829500	conserved Plasmodium protein	exon 3 segment 1
PF3D7_0830500	tryptophan/threonine-rich antigen (TryThrA)	
PF3D7_0831700	heat shock protein 70 (HSP70-x)	
PF3D7_0900200	rifin (RIF)	exon 2 segment 1
PF3D7_0902200	serine/threonine protein kinase, FIKK family (FIKK9.3)	
PF3D7_0903000	conserved protein	
PF3D7_0903000	conserved protein	
PF3D7_0903500	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0903500	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0903800	LCCL domain-containing protein (CCp4)	exon 2 segment 1
PF3D7_0903800	LCCL domain-containing protein (CCp4)	exon 2 segment 2
PF3D7_0905400	high molecular weight rhoptry protein 3 (RhopH3)	exon 7 segment 1
PF3D7_0907200	GTPase activator, putative	exon 1 segment 2

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_0909500	subpellicular microtubule protein 1, putative (SPM1)	
PF3D7_0909500	subpellicular microtubule protein 1, putative (SPM1)	
PF3D7_0911700	GTP binding protein, putative	
PF3D7_0912500	conserved Plasmodium protein	
PF3D7_0912800	tRNA 1-methyladenosine methyltransferase subunit, putative	
PF3D7_0915400	6-phosphofructokinase (PFK9)	exon 1 segment 1
PF3D7_0915400	6-phosphofructokinase (PFK9)	exon 1 segment 1
PF3D7_0917500	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0918900	gamma-glutamylcysteine synthetase (gammaGCS)	exon 1 segment 2
PF3D7_0919300	thioredoxin, putative	exon 3 segment 1
PF3D7_0919300	thioredoxin, putative	exon 3 segment 1
PF3D7_0920300	conserved Plasmodium protein	
PF3D7_0922100	ubiquitin-like protein, putative	exon 1 segment 2
PF3D7_0927400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_0927400	conserved Plasmodium protein	exon 2 segment 1
PF3D7_0927400	conserved Plasmodium protein	exon 3 segment 1
PF3D7_0930300	merozoite surface protein 1 (MSP1)	
PF3D7_0930300	merozoite surface protein 1 (MSP1)	
PF3D7_0930300	merozoite surface protein 1 (MSP1)	
PF3D7_0930300	merozoite surface protein 1 (MSP1)	
PF3D7_0930300	merozoite surface protein 1 (MSP1)	
PF3D7_0930300	merozoite surface protein 1 (MSP1)	segment 1
PF3D7_0930300	merozoite surface protein 1 (MSP1)	segment 2
PF3D7_0930800	conserved Plasmodium membrane protein	exon 2 segment 1
PF3D7_0930800	conserved Plasmodium membrane protein	exon 2 segment 2
PF3D7_0930800	conserved Plasmodium membrane protein	exon 2 segment 3
PF3D7_0930800	conserved Plasmodium membrane protein	exon 2 segment 4
PF3D7_0930800	conserved Plasmodium membrane protein	exon 2 segment 5
PF3D7_0931300	asparagine-rich antigen, putative	
PF3D7_0931300	asparagine-rich antigen, putative	
PF3D7_0933100	conserved Plasmodium protein	
PF3D7_0933100	conserved Plasmodium protein	
PF3D7_0933900	conserved Plasmodium protein	
PF3D7_0935600	gametocytogenesis-implicated protein (GIG)	
PF3D7_0935900	ring-exported protein 1 (REX1)	exon 2 segment 1
PF3D7_0936300	ring-exported protein 3 (REX3)	
PF3D7_0937000	Plasmodium exported protein (PHISTb)	
PF3D7_1001300	Plasmodium exported protein (PHISTa)	
PF3D7_1001500	early transcribed membrane protein 10.1 (ETRAMP10)	
PF3D7_1002000	Plasmodium exported protein (hyp2)	
PF3D7_1002100	PF70 protein (PF70)	
PF3D7_1002800	DnaJ protein, putative	exon 1 segment 1
PF3D7_1002800	DnaJ protein, putative	exon 1 segment 2
PF3D7_1003800	U5 small nuclear ribonuclear protein, putative	exon 1 segment 2
PF3D7_1004800	ADP/ATP carrier protein, putative	exon 1 segment 1
PF3D7_1007700	transcription factor with AP2 domain(s) (ApiAP2)	exon 1 segment 2
PF3D7_1008100	conserved Plasmodium protein	segment 1
PF3D7_1008700	tubulin beta chain	exon 3 segment 1
PF3D7_1009100	conserved Plasmodium membrane protein	
PF3D7_1009100	conserved Plasmodium membrane protein	

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1009500	metallopeptidase, putative	exon 3
PF3D7_1009500	metallopeptidase, putative	exon 3
PF3D7_1012300	ubiquinol-cytochrome c reductase complex subunit, putative	exon 2 segment 1
PF3D7_1014100	conserved Plasmodium protein	segment 1
PF3D7_1014100	conserved Plasmodium protein	segment 2
PF3D7_1015100	conserved protein	
PF3D7_1016500	Plasmodium exported protein (PHISTc)	exon 1 segment 1
PF3D7_1016500	Plasmodium exported protein (PHISTc)	exon 2 segment 1
PF3D7_1016900	early transcribed membrane protein 10.3 (ETRAMP10.3)	exon 1 segment 1
PF3D7_1018000	tRNA pseudouridine synthase, putative	
PF3D7_1018300	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1018600; PF3D7_1018500	PHF5-like protein, putative; conserved Plasmodium protein	exon 1 segment 2
PF3D7_1019100	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1019100	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1020800	dihydrolipoamide acyltransferase component E2 (DLAT)	
PF3D7_1020800	dihydrolipoamide acyltransferase component E2 (DLAT)	
PF3D7_1022000	RNA binding protein, putative	exon 2
PF3D7_1022200; PF3D7_1022100	conserved Plasmodium protein; conserved Plasmodium membrane protein	exon 1 segment 1
PF3D7_1022200; PF3D7_1022100	conserved Plasmodium protein; conserved Plasmodium membrane protein	exon 2 segment 1
PF3D7_1022200; PF3D7_1022100	conserved Plasmodium protein; conserved Plasmodium membrane protein	exon 4 segment 1
PF3D7_1022200; PF3D7_1022100	conserved Plasmodium protein; conserved Plasmodium membrane protein	exon 5 segment 1
PF3D7_1023000	conserved Plasmodium membrane protein	
PF3D7_1023100	dynein heavy chain, putative	exon 1 segment 3
PF3D7_1023100	dynein heavy chain, putative	exon 1 segment 4
PF3D7_1024800	conserved Plasmodium protein	exon 2 segment 2
PF3D7_1025500	conserved Plasmodium protein	
PF3D7_1028900	conserved Plasmodium protein	
PF3D7_1033200	early transcribed membrane protein 10.2 (ETRAMP10.2)	exon 1 segment 1
PF3D7_1035300	glutamate-rich protein (GLURP)	
PF3D7_1035300	glutamate-rich protein (GLURP)	
PF3D7_1035400	merozoite surface protein 3 (MSP3)	
PF3D7_1035400	merozoite surface protein 3 (MSP3)	
PF3D7_1035400	merozoite surface protein 3 (MSP3)	
PF3D7_1035400	merozoite surface protein 3 (MSP3)	
PF3D7_1035500	merozoite surface protein 6 (MSP6)	
PF3D7_1035600	merozoite surface protein (H101)	
PF3D7_1035700	duffy binding-like merozoite surface protein (DBLMSP)	exon 1 segment 1
PF3D7_1035800	probable protein (M712)	
PF3D7_1035900	probable protein (M566)	
PF3D7_1036000	merozoite surface protein (MSP11)	
PF3D7_1036300	merozoite surface protein (DBLMSP2)	
PF3D7_1036400	liver stage antigen 1 (LSA1)	
PF3D7_1036400	liver stage antigen 1 (LSA1)	exon 1
PF3D7_1036400	liver stage antigen 1 (LSA1)	exon 2 segment 1
PF3D7_1036400	liver stage antigen 1 (LSA1)	exon 2 segment 2

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1037600	DNA repair helicase rad25, putative	exon 2 segment 1
PF3D7_1037600	DNA repair helicase rad25, putative	exon 3 segment 1
PF3D7_1038500	Plasmodium exported protein	
PF3D7_1040800	rifin (RIF)	exon 2 segment 1
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 1
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 2
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 3
PF3D7_1100200	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_1100800	Pfmc-2TM Maurer's cleft two transmembrane protein (MC-2TM)	
PF3D7_1102300	Plasmodium exported protein	
PF3D7_1102500	Plasmodium exported protein (PHISTb) (GEXP02)	exon 2 segment 1
PF3D7_1106300	exonuclease, putative	
PF3D7_1107100	nucleic acid binding protein, putative	exon 6 segment 1
PF3D7_1112300	conserved Plasmodium protein	exon 1
PF3D7_1113500	GTP binding protein, putative	
PF3D7_1113600	Rpr2, RNAse P, putative	
PF3D7_1114900	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1115200	SET domain protein, putative (SET7)	
PF3D7_1122400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	exon 1 segment 1
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	exon 1 segment 2
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	exon 2 segment 1
PF3D7_1122800	calcium-dependent protein kinase 6 (CDPK6)	exon 3 segment 1
PF3D7_1123400	translation elongation factor EF-1, subunit alpha, putative	exon 1
PF3D7_1124500	pyruvate dehydrogenase E1 alpha subunit (pdhA)	
PF3D7_1124500	pyruvate dehydrogenase E1 alpha subunit (pdhA)	exon 1 segment 1
PF3D7_1125800	kelch protein, putative	
PF3D7_1126000	threonine--tRNA ligase (ThrRS)	exon 1 segment 1
PF3D7_1128100	cochaperone prefoldin complex subunit, putative	
PF3D7_1129100	parasitophorous vacuolar protein 1 (PV1)	exon 1 segment 1
PF3D7_1129600	phosphatidylinositol-4-phosphate-5-kinase, putative	exon 1 segment 2
PF3D7_1130000	N-acetyl glucosamine phosphate mutase, putative	
PF3D7_1131400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1131600	conserved Plasmodium protein	segment 1
PF3D7_1131600	conserved Plasmodium protein	segment 2
PF3D7_1131600	conserved Plasmodium protein	segment 3
PF3D7_1132400	conserved Plasmodium membrane protein	exon 2 segment 1
PF3D7_1132400	conserved Plasmodium membrane protein	exon 2 segment 2
PF3D7_1133400	apical membrane antigen 1 (AMA1)	
PF3D7_1133400	apical membrane antigen 1 (AMA1)	
PF3D7_1133400	apical membrane antigen 1 (AMA1)	
PF3D7_1133400	apical membrane antigen 1 (AMA1)	
PF3D7_1133400	apical membrane antigen 1 (AMA1)	
PF3D7_1134500	alpha/beta hydrolase, putative	segment 1
PF3D7_1134500	alpha/beta hydrolase, putative	segment 1
PF3D7_1139300	transcription factor with AP2 domain(s) (ApiAP2)	exon 2 segment 3
PF3D7_1140800	conserved Plasmodium protein	
PF3D7_1140900	conserved Plasmodium protein	
PF3D7_1142300	conserved Plasmodium membrane protein	exon 2 segment 1

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1142300	conserved Plasmodium membrane protein	exon 2 segment 2
PF3D7_1142600	60S ribosomal protein L35ae, putative	exon 1 segment 1
PF3D7_1146200	conserved Plasmodium protein	
PF3D7_1147000	sporozoite asparagine-rich protein (SLARP)	exon 1 segment 1
PF3D7_1147000	sporozoite asparagine-rich protein (SLARP)	exon 1 segment 2
PF3D7_1147000	sporozoite asparagine-rich protein (SLARP)	exon 1 segment 3
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	exon 1 segment 1
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	segment 1
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	segment 4
PF3D7_1149000	antigen 332, DBL-like protein (Pf332)	segment 6
PF3D7_1149200	ring-infected erythrocyte surface antigen	exon 2 segment 1
PF3D7_1149200	ring-infected erythrocyte surface antigen	exon 2 segment 2
PF3D7_1200300	rifin (RIF)	exon 2 segment 1
PF3D7_1207400	conserved Plasmodium protein	
PF3D7_1207400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1207400	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1207500	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1207500	conserved Plasmodium protein	exon 3 segment 1
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	exon 1 segment 1
PF3D7_1208200	cysteine repeat modular protein 3 (CRMP3)	exon 3 segment 1
PF3D7_1208800	zinc finger protein, putative	exon 1 segment 1
PF3D7_1208800	zinc finger protein, putative	exon 1 segment 2
PF3D7_1209400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1211000	kinesin-like protein, putative	exon 3 segment 1
PF3D7_1211300	DNA helicase MCM8, putative (MCM8)	exon 1 segment 1
PF3D7_1211300	DNA helicase MCM8, putative (MCM8)	exon 2 segment 1
PF3D7_1211900	non-SERCA-type Ca2 -transporting P-ATPase (ATP4)	exon 1 segment 1
PF3D7_1212900	bromodomain protein, putative	exon 1 segment 2
PF3D7_1213400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1213400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1213500	integral membrane protein GPR180, putative	
PF3D7_1213500	integral membrane protein GPR180, putative	
PF3D7_1216400	conserved Plasmodium membrane protein	
PF3D7_1216500	male development gene 1 (MDV1)	exon 1 segment 1
PF3D7_1216600	cell traversal protein for ookinetes and sporozoites (CelTOS)	
PF3D7_1216600	cell traversal protein for ookinetes and sporozoites (CelTOS)	exon 1 segment 1
PF3D7_1218400	triose or hexose phosphate/phosphate translocator, putative	
PF3D7_1219700	raf kinase inhibitor (RKIP)	exon 1 segment 1
PF3D7_1221400	inner membrane complex protein 1h, putative (IMC1h)	exon 1 segment 1
PF3D7_1222300	endoplasmin, putative (GRP94)	exon 1 segment 1
PF3D7_1222400	transcription factor with AP2 domain(s) (ApiAP2)	exon 1 segment 2
PF3D7_1223300	DNA gyrase subunit A (GyrA)	
PF3D7_1225900	conserved Plasmodium protein	
PF3D7_1226000	conserved Plasmodium protein	exon 1
PF3D7_1228400	conserved Plasmodium protein	segment 1
PF3D7_1228400	conserved Plasmodium protein	segment 2
PF3D7_1228600	merozoite surface protein 9 (MSP9)	exon 1 segment 1
PF3D7_1229000	conserved Plasmodium membrane protein	exon 1 segment 1
PF3D7_1229000	conserved Plasmodium membrane protein	exon 1 segment 2
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	exon 1 segment 1

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	exon 1 segment 2
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	exon 1 segment 3
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	segment 1
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	segment 2
PF3D7_1229100	ABC transporter, (CT family) (MRP2)	segment 3
PF3D7_1229300	conserved Plasmodium protein	
PF3D7_1230100	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1230100	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1232100	60 kDa chaperonin (CPN60)	exon 1
PF3D7_1233300	pentatricopeptide repeat protein, putative	segment 1
PF3D7_1233300	pentatricopeptide repeat protein, putative	segment 2
PF3D7_1233600	asparagine and aspartate rich protein 1 (AARP1)	exon 1 segment 3
PF3D7_1235400	tetQ family GTPase, putative	exon 1 segment 2
PF3D7_1236100	clustered-asparagine-rich protein	exon 2 segment 1
PF3D7_1243400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1244000	glucose inhibited division protein a homologue, putative	
PF3D7_1244100	N-alpha-acetyltransferase 15, NatA auxiliary subunit, putative	exon 1 segment 2
PF3D7_1244400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1244400	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1246000	conserved Plasmodium protein	
PF3D7_1247100	conserved Plasmodium protein	
PF3D7_1247200	conserved Plasmodium protein	
PF3D7_1249000	conserved Plasmodium membrane protein	
PF3D7_1250200	conserved Plasmodium membrane protein	
PF3D7_1250200	conserved Plasmodium membrane protein	exon 1
PF3D7_1250400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1250900	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1252100	rhoptry neck protein 3 (RON3)	exon 8 segment 2
PF3D7_1252400	reticulocyte binding protein homologue 3, pseudogene (RH3)	exon 3 segment 1
PF3D7_1252500	Plasmodium exported protein	
PF3D7_1254100	stevor	exon 2 segment 1
PF3D7_1254100	stevor	exon 2 segment 1
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 1
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 1 segment 2
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_1300300	erythrocyte membrane protein 1, PfEMP1 (VAR)	exon 2 segment 1
PF3D7_1300600	rifin (RIF)	exon 2 segment 1
PF3D7_1301600	erythrocyte binding antigen-140 (EBA140)	
PF3D7_1302000	Plasmodium exported protein	
PF3D7_1302800	40S ribosomal protein S7, putative	exon 1
PF3D7_1303400	conserved Plasmodium protein	
PF3D7_1304600	conserved Plasmodium protein	
PF3D7_1306500	MORN repeat protein, putative	exon 1 segment 1
PF3D7_1308700	conserved Plasmodium protein	
PF3D7_1311600	conserved Plasmodium protein	
PF3D7_1311800	M1-family alanyl aminopeptidase (M1AAP)	exon 1 segment 1
PF3D7_1315200	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1315200	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1318100	ferredoxin, putative	
PF3D7_1319400	conserved Plasmodium protein	

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1319900	conserved Plasmodium protein	segment 1
PF3D7_1320800	dihydrolipamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex	exon 3
PF3D7_1321100	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1321100	conserved Plasmodium protein	exon 3 segment 1
PF3D7_1321600	phosphodiesterase gamma, putative (PDEgamma)	
PF3D7_1322200	conserved Plasmodium protein	exon 4 segment 1
PF3D7_1325900	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1328500	alpha/beta-hydrolase, putative	exon 1 segment 2
PF3D7_1329100	myosin C (MyoC)	exon 3 segment 3
PF3D7_1329100	myosin C (MyoC)	exon 3 segment 3
PF3D7_1331000	protein kinase, putative	
PF3D7_1332100	conserved Plasmodium membrane protein	
PF3D7_1332900	isoleucine--tRNA ligase, putative	exon 1 segment 1
PF3D7_1333100	conserved Plasmodium protein	
PF3D7_1333200	ubiquitin-activating enzyme (UBA1)	segment 1
PF3D7_1333200	ubiquitin-activating enzyme (UBA1)	segment 2
PF3D7_1334200	chaperone binding protein, putative	exon 1 segment 1
PF3D7_1334300	MSP7-like protein (MSRP5)	
PF3D7_1334600	MSP7-like protein (MSRP3)	
PF3D7_1334600	MSP7-like protein (MSRP3)	exon 1 segment 1
PF3D7_1334600	MSP7-like protein (MSRP3)	exon 2 segment 1
PF3D7_1334800	MSP7-like protein (MSRP2)	
PF3D7_1335100	merozoite surface protein 7 (MSP7)	
PF3D7_1335100	merozoite surface protein 7 (MSP7)	
PF3D7_1335300	reticulocyte binding protein 2 homologue b (RH2b)	exon 1 segment 1
PF3D7_1335300	reticulocyte binding protein 2 homologue b (RH2b)	exon 1 segment 2
PF3D7_1335400	reticulocyte binding protein 2 homologue a (RH2a)	
PF3D7_1335400	reticulocyte binding protein 2 homologue a (RH2a)	
PF3D7_1335900	sporozoite surface protein 2 (TRAP)	
PF3D7_1335900	sporozoite surface protein 2 (TRAP)	exon 1 segment 1
PF3D7_1337300	exoribonuclease, putative	
PF3D7_1337300	exoribonuclease, putative	
PF3D7_1339300	conserved Plasmodium protein	
PF3D7_1339600	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1341800	conserved Plasmodium protein	
PF3D7_1342500	sporozoite protein essential for cell traversal (SPECT)	exon 5 segment 1
PF3D7_1342500	sporozoite protein essential for cell traversal (SPECT)	exon 5 segment 1
PF3D7_1344500	conserved Plasmodium protein	
PF3D7_1346000	dynactin subunit 2, putative	exon 1 segment 1
PF3D7_1346400	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1346700	6-cysteine protein (P48/45)	
PF3D7_1346700	6-cysteine protein (P48/45)	
PF3D7_1350600	conserved Plasmodium protein	
PF3D7_1351900	conserved Plasmodium protein	
PF3D7_1353100	Plasmodium exported protein	exon 1 segment 1
PF3D7_1353100	Plasmodium exported protein	exon 2 segment 1
PF3D7_1354200	inositol-polyphosphate 5-phosphatase, putative	
PF3D7_1356800	serine/threonine protein kinase, putative (ARK3)	exon 1 segment 2
PF3D7_1356800	serine/threonine protein kinase, putative (ARK3)	exon 1 segment 5

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1359700	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1359700	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1359700	conserved Plasmodium protein	exon 1 segment 4
PF3D7_1360400	conserved Plasmodium protein	
PF3D7_1363700	conserved Plasmodium protein	
PF3D7_1364200	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1364300	pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16 (PRP16)	exon 1 segment 1
PF3D7_1364400	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1366600	signal recognition particle receptor alpha subunit (SRPR-alpha)	
PF3D7_1366900	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1367800	secreted ookinete protein, putative (PSOP2)	exon 1 segment 1
PF3D7_1367800	secreted ookinete protein, putative (PSOP2)	exon 1 segment 2
PF3D7_1401400	early transcribed membrane protein 14.1 (ETRAMP14)	
PF3D7_1410400	rhoptry-associated protein 1 (RAP1)	exon 1 segment 1
PF3D7_1411700	conserved protein	
PF3D7_1413200	conserved Plasmodium protein	exon 4 segment 1
PF3D7_1414400	serine/threonine protein phosphatase (PP1)	exon 1 segment 1
PF3D7_1414500	atypical protein kinase, ABC-1 family, putative (ABCk2)	exon 4 segment 1
PF3D7_1414500	atypical protein kinase, ABC-1 family, putative (ABCk2)	exon 4 segment 2
PF3D7_1414500	atypical protein kinase, ABC-1 family, putative (ABCk2)	exon 5 segment 2
PF3D7_1414700	ubiquitin carboxyl-terminal hydrolase, putative	segment 1
PF3D7_1414700	ubiquitin carboxyl-terminal hydrolase, putative	segment 2
PF3D7_1415700	serine C-palmitoyltransferase, putative	
PF3D7_1416600	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1416600	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1417000	conserved Plasmodium protein	
PF3D7_1417200	NOT family protein, putative	exon 1 segment 1
PF3D7_1418100	liver specific protein 1, putative (LISP1)	segment 3
PF3D7_1419400	conserved Plasmodium membrane protein	exon 1 segment 1
PF3D7_1419400	conserved Plasmodium membrane protein	exon 1 segment 2
PF3D7_1426700	phosphoenolpyruvate carboxylase, putative (PEPC)	exon 1 segment 1
PF3D7_1428200	metabolite/drug transporter, putative	
PF3D7_1429100	apicoplast ribosomal protein L15 precursor, putative	
PF3D7_1429100	apicoplast ribosomal protein L15 precursor, putative	
PF3D7_1429500	diphthamide synthesis protein, putative	
PF3D7_1433400	conserved Plasmodium membrane protein	exon 2 segment 3
PF3D7_1433400	conserved Plasmodium membrane protein	exon 2 segment 3
PF3D7_1436300	translocon component PTEX150 (PTEX150)	exon 1 segment 1
PF3D7_1437200	ribonucleoside-diphosphate reductase, large subunit, putative	exon 1
PF3D7_1438100	secretory complex protein 62 (SEC62)	
PF3D7_1439100	DEAD/DEAH box helicase, putative	exon 1 segment 2
PF3D7_1439800	vesicle-associated membrane protein, putative	exon 1
PF3D7_1441300	serine/threonine protein kinase, putative	exon 3 segment 1
PF3D7_1441300	serine/threonine protein kinase, putative	exon 4 segment 1
PF3D7_1441300	serine/threonine protein kinase, putative	exon 5 segment 2
PF3D7_1442700	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1442700	conserved Plasmodium protein	exon 2 segment 2

Appendix B. 655 *P. falciparum* antigens on the Ugandan protein microarray (Chapter 2) meeting the inclusion criteria (continued).

Gene ID	Description	Exon & Segment
PF3D7_1444100	conserved Plasmodium protein	exon 6 segment 1
PF3D7_1446400	pyruvate dehydrogenase E1 beta subunit (pdhB)	
PF3D7_1447500	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1448300	conserved Plasmodium protein	segment 2
PF3D7_1448500	conserved Plasmodium protein	exon 1 segment 4
PF3D7_1452000	rhoptry neck protein 2 (RON2)	segment 1
PF3D7_1452000	rhoptry neck protein 2 (RON2)	segment 2
PF3D7_1454800	conserved Plasmodium protein	
PF3D7_1456500	conserved Plasmodium protein	segment 2
PF3D7_1462100	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1462100	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1462300	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1462400	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1462400	conserved Plasmodium protein	exon 1 segment 2
PF3D7_1462400	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1465800	dynein beta chain, putative	exon 4 segment 2
PF3D7_1466200	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1466300	26S proteasome regulatory subunit, putative	exon 1 segment 1
PF3D7_1467900	rab GTPase activator, putative	exon 3 segment 2
PF3D7_1468100	conserved Plasmodium protein	exon 2 segment 1
PF3D7_1469600	biotin carboxylase subunit of acetyl CoA carboxylase, putative (ACC1)	exon 1 segment 4
PF3D7_1470100	conserved Plasmodium protein	exon 1 segment 1
PF3D7_1470100	conserved Plasmodium protein	exon 1 segment 3
PF3D7_1471100	exported protein 2 (EXP2)	exon 3 segment 1
PF3D7_1471400	diacylglycerol kinase, putative	exon 2 segment 1
PF3D7_1472300	conserved Plasmodium membrane protein	
PF3D7_1472300	conserved Plasmodium membrane protein	exon 2
PF3D7_1473400	conserved Plasmodium protein	
PF3D7_1475900	conserved Plasmodium protein	
PF3D7_1476400	serine/threonine protein kinase, FIKK family, pseudogene (FIKK14)	
PF3D7_1476600	Plasmodium exported protein	
PF3D7_1476600	Plasmodium exported protein	exon 2
PF3D7_1477000	Plasmodium exported protein (hyp17), pseudogene	
PF3D7_1477500	Plasmodium exported protein (PHISTb)	
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	exon 1
PF3D7_1477900	acyl-CoA synthetase, pseudogene (ACS1b)	exon 3
PF3D7_1479000	acyl-CoA synthetase (ACS1a)	