A randomized controlled phase 1 trial of malaria transmission-blocking vaccines Pfs230D1-EPA and Pfs25-EPA in Alhydrogel® in healthy Malian adults

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1 CONTRIBUTIONS TO THE TRIAL

1.1 Data and Safety Monitoring Board (DSMB)

The NIAID Intramural DSMB served as the study's safety monitoring board. As outlined in the protocol prior to study start, initial DSMB meeting held prior to study start (February 2014) with structured interim reviews (December 2014, February 2015, March 2015, May 2015, June 2016, December 2016) with an additional subsequent review occurring in March 2017 to review study results.

1.2 Study Oversight and Funding

The study protocol was approved by the ethical review board in Mali (Nº 2015/16/CE/FMPOS), NIH/NIAID (#15-I-0044) institutional review board, Mali national regulatory authority, and conducted under FDA IND 16251. The trial was undertaken in accordance with the provision of the Good Clinical Practice Guidelines and in alignment with institutional procedures and guidelines. The MRTC at the Mali-NIAID International Center of Excellence in Research undertook the clinical conduct of the study in collaboration with the Laboratory of Malaria Immunology and Vaccinology (LMIV) team. Office of Clinical Research Policy and Regulatory Operations (OCRPRO) from the NIAID intramural research program was the study Sponsor and coordinated regulatory submissions and communication to the US FDA and contracted an independent monitor for clinical monitoring oversight. NIAID Data and Safety Monitoring Board was closely involved in the progress and active review of the study as noted in **Section 1.1**. Professor Mamadou Dembele, MD served as the Independent Safety Monitor (ISM) in Mali and was available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. He attended DSMB meetings and received safety summary reporting during the course of the study.

1.3 Acknowledgements

We thank Professor Mamadou Dembele for his expert advice and consultation during the course and the members of the NIAID Data and Safety Monitoring Board for their insight, discussion, and recommendations. Also thank you to the FMPOS Ethics Committee, NIAID IRB, Malian Ministry of Health and Public Hygiene, and Malian Department of Pharmacy and Medicine.

We are grateful to the USTTB Rector Adama Keita and his office and NIAID Division of Intramural Research (DIR) for their constant support of our collaborative projects in Mali.

We thank Mohamed Keita and Hammadoun Dicko for their collaboration as the emergency physicians and Bourama Kane, Mamadou Ouane and Belco Maiga for their skills and

participation as the vaccinators during the course of the study. Additionally, we are grateful to Sory Ibrahim Traore, the Bancoumana community health facility physician for his cooperation.

This study could not have been successful without the significant support from the MRTC Entomology team.

We are grateful to the MRTC Clinical Laboratory Team which includes Professor Boubacar Traore, Moussa Lamine Diakite, Sintry Sanogo, Sekouba Keita, Boucary Ouologuem, Souleymane Traore.

We are grateful to the following at LMIV: the Clinical Trials Team which includes Regina White; Project Management Team, which includes Sharon Wong-Madden; and Logistics and Sample Management Team which includes Rhea Stevens. We thank Vu Nguyen, Richard Shimp, Jr., Raul Herrera, and Karine Reiter for oversight of the production and release of bulk conjugated vaccine. We also thank Holly McClellan and Weili Dai for their assistance in vaccine quality control evaluation. We are grateful for the work by Emily Kelnhofer and Ashley McCormack on the SMFA results. We also would like to thank the O'Connell lab for completing the stool qPCR assays for the main cohort.

In addition, this work could not have been completed without the support of the NIAID team based in Bamako Mali, including Richard Sakai, Souleymane Karambe, and Mame Niang, and support from NIAID/DIR including Joseph Shott.

We thank OCRPRO and Office of Cyber Infrastructure and Computational Biology at the NIH for providing assistance in the development and finalization of the case report forms and database, including Kevin Newell, Michael Duvenhage, Michael Holdsworth, Lisa Hoopengardner, Diane Rock, and local support in Mali from MRTC for data entry and validation (Moussa Tienta and Siriman Traore).

We thank OCRPRO for providing external monitoring services for this study and ongoing support and guidance to our NIAID/MRTC programs, specifically Delphine Yamadjako, Lisa Giebeig, Shelly Simpson, and Susan Vogel.

2 METHODS

2.1 Enrolment

Following enrolment, vaccination, and safety review of US subjects, summarized previously in Healy et al.,[1] safety cohort subjects (5/arm; n=25) were enrolled in a double-blind, comparator-controlled pilot study to receive single vaccinations (Pfs25, Pfs230D1, TWINRIX) on days 0 and 28, followed by a separate cohort receiving co-administered vaccinations (Pfs25+Pfs230D1, TWINRIX + normal saline on the same schedule; pilot-safety cohort participants were followed

for 6 months post dose 2 for safety and immunogenicity. Subjects were then enrolled into the main double-blind, comparator-controlled study (n=200) and divided into 4 arms: Pfs25 + normal saline; Pfs230D1 + normal saline; Pfs25 + Pfs230D1; and comparator (TWINRIX + normal saline for dose 1, 2, 3, Menactra + normal saline for dose 4).

Comparator vaccines (TWINRIX, Menactra) were offered to subjects after unblinding (pilot-safety cohort: study day 196 (6 months post dose 2); main cohort: study day 730 (6 months post dose 4). An optional large volume blood draw follow-up visit (study day 910), approximately 12 months post dose 4, for additional serological analysis and characterization of B cell receptor usage was offered to those individuals with high vaccine specific antibody responses.

2.2 Randomization and Masking

The study was to be enrolled in the following manner in a double-blind, en-bloc randomization within each of the following groups (**Figure S1**):

- Pfs25-EPA/Alhydrogel®, 16µg (n=5); Pfs230D1-EPA/Alhydrogel®, 15µg (n=5), TWINRIX (n=5) – total 15
- Pfs25-EPA/Alhydrogel®, 16µg + Pfs230D1-EPA/Alhydrogel®, 15µg (n=5); TWINRIX + normal saline (n=5) – total 10
- Pfs25-EPA/Alhydrogel®, 47µg (n=50); Pfs230D1-EPA/Alhydrogel®, 40µg (n=50); Pfs25-EPA/Alhydrogel®, 47µg + Pfs230D1-EPA/Alhydrogel®, 40µg (n=50); TWINRIX/Menactra + normal saline (n=50) – total 200

As noted in the main text, due to one subject randomized to Pfs230D1, 40µg + normal saline being erroneously administered comparator for vaccination #1; Pfs230D1, 40µg + normal saline started with n=49 while TWINRX/Menactra + normal saline started with n=51.

2.3 Vaccines

Vaccines were administered as intramuscular injections into the deltoid muscle. Arms were alternated with successive vaccinations if a single vaccination was given. If simultaneous vaccinations were administered (two individual vaccinations at the same time), each vaccine was drawn up and delivered separately, in alternate arms; the arm of the subject that receives the normal saline were alternated with successive vaccinations. When choosing an arm for the vaccine injection, clinicians considered whether there was an arm injury, local skin problems such as scarring or rash, or significant tattoo that precluded administering the injection or would have interfered with evaluating the arm after injection. In keeping with MRTC practices and procedures, and good medical practice, acute medical care was provided to subjects for any immediate allergic reactions or other injury resulting from participation in this research study.

Due to the variance in volume of the study product (Pfs25 and Pfs230D1 in comparison to the control vaccines (TWINRIX®, Menactra®, and normal saline), opaque tape was wrapped around the vaccine syringe(s) when being administered.

Contraindication to vaccination included: hypersensitivity reaction following administration of the study vaccine, positive pregnancy test prior to vaccination, or a safety concern determined by the study investigator. Vaccination was deferred when oral temperature was >37·5°C at the time of vaccination or any other condition that in the opinion of the Investigator posed a threat or may complicate interpretation of safety of the vaccine post immunization.

2.3.1 Pfs25M-EPA/Alhydrogel®

Each Pfs25 vaccine vial contained 78 µg/mL conjugated Pfs25, 78 µg/mL conjugated EPA, and 1600 µg/mL Alhydrogel in a volume of 0·8 mL.[2]

The Pfs25M-EPA/Alhydrogel vaccine was provided as a single-use vial. A 0·2-mL volume is administered for delivery of 16 µg conjugated Pfs25M, 16 µg conjugated EPA, and 320 µg Alhydrogel. A 0·6-mL volume is administered for delivery of 47 µg conjugated Pfs25M, 47 µg conjugated EPA, and 960 µg Alhydrogel. The vaccine can be drawn up into the syringe up to 5 hours prior to administration and will be mixed by hand before injection to ensure resuspension.

2.3.2 Pfs230D1M-EPA/Alhydrogel®

Each Pfs230D1 vaccine vial contained 50 µg/mL conjugated Pfs230D1, 49 µg/mL conjugated EPA and 1600 µg/mL Alhydrogel in a volume of 1·0 mL.

The Pfs230D1M-EPA/Alhydrogel vaccine was formulated in cGMP compliance in July 2014 and will be provided as a single-use vial. A 0·1-mL volume is administered for delivery of 5 µg conjugated Pfs230D1M, 5 µg conjugated EPA, and 160 µg Alhydrogel. A 0·3-mL volume is administered for delivery of 15 µg conjugated Pfs230D1M, 15 µg conjugated EPA, and 480 µg Alhydrogel. A 0·8-mL volume is administered for delivery of 40 µg conjugated Pfs230D1M, 40 µg conjugated EPA, and 1280 µg Alhydrogel. The vaccine can be drawn up into the syringe up to 5 hours prior to administration and will be mixed by hand before injection to ensure resuspension

2.3.3 TWINRIX® (Hepatitis A & Hepatitis B [Recombinant] Vaccine)

TWINRIX (Hepatitis A & Hepatitis B [Recombinant] Vaccine; GlaxoSmithKline) is a bivalent vaccine containing the antigenic components used in producing HAVRIX® (Hepatitis A Vaccine; GlaxoSmithKline) and ENGERIX-B (Hepatitis B Vaccine [Recombinant]; GlaxoSmithKline). TWINRIX is a sterile suspension for intramuscular administrations that contains inactivated hepatitis A virus (strain HM175) and noninfectious hepatitis B virus surface antigen (HBsAg). The hepatitis A virus is propagated in MRC-5 human diploid cells and

inactivated with formalin. The purified HBsAg is obtained by culturing genetically engineered *Saccharomyces cerevisiae* yeast cells, which carry the surface antigen gene of the hepatitis B virus. Bulk preparations of each antigen are adsorbed separately onto aluminum salts and then pooled during formulation. A 1 mL dose of vaccine contains 720 ELISA units of inactivated hepatitis A virus and 20 mcg of recombinant HBsAg protein, 0·45 mg of aluminum in the form of aluminum phosphate and aluminum hydroxide as adjuvants, amino acids, sodium chloride, phosphate buffer, polysorbate 20, and water for injection. The vaccine is manufactured by GlaxoSmithKline. TWINRIX is FDA approved for the active immunization against disease caused by hepatitis A virus and infection by all known subtypes of hepatitis B virus in nonpregnant adults 18 years of age and older at the standard dosing of 3 vaccinations given at 0-, 1-, and 6-month schedule.

2.3.4 Menactra® (Meningococcal Vaccine)

Menactra (Sanofi Pasteur) is a sterile, intramuscularly administered vaccine that contains Neisseria meningitidis serogroup A, C, Y, and W-135 capsular polysaccharide antigens individually conjugated to diphtheria toxoid protein. No preservative or adjuvant is added during the manufacturing process. Menactra is FDA approved for active immunization to prevent invasive meningococcal disease caused by Neisseria meningitidis serogroups A, C, Y, and W-135 (but does not protect against serotype B) for use in individuals 9 months through 55 years of age. A single dose (0·5 mL) is recommended for those individuals 18 to 45 years of age and otherwise healthy who are at increased risk for meningococcal disease (e.g., individuals in an epidemic or highly endemic country such as Mali).

2.3.5 Normal Saline

Sterile isotonic (0·9%) normal saline was commercially procured in the US and shipped to Mali at ambient temperature. Normal saline will be administered in a 1mL dose as an intramuscular injection.

2.4 Inclusion/Exclusion Criteria

2.4.1 Inclusion Criteria

All of the following criteria were fulfilled for a volunteer participating in this trial:

1. Age ≥ 18 and ≤ 50 years.
2. Available for the duration of the trial.
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
4. In good general health and without clinically significant medical history.

5. Females of childbearing potential were willing to use reliable contraception (as defined below) from 21 days prior to Study Day 0 to 3 months after the last vaccination.
    - Reliable methods of birth control included **one** of the following: confirmed pharmacologic contraceptives (parenteral) delivery; intrauterine or implantable device.
    - Reliable methods of birth control included concurrent use of a pharmacologic and a barrier method, i.e. **two** of the following: confirmed pharmacologic contraceptives (oral, transdermal) delivery or vaginal ring **AND** condoms with spermicide or diaphragm with spermicide.
    - Abstinence of potentially reproductive sexual activity.
    - Non-childbearing women were required to report date of last menstrual period, history of surgical sterility (i.e. tubal ligation, hysterectomy) or premature ovarian insufficiency (POI), and had a baseline urine or serum pregnancy test performed.
6. Willingness to have blood samples stored for future research.
7. Willingness to undergo direct skin feeds.
8. Known resident of Bancoumana or surrounding area.

2.4.2 Exclusion Criteria

A subject was excluded from participating in this trial if any **one** of the following criteria was fulfilled:

1. Pregnancy as determined by a positive urine or serum human choriogonadotropin (β-hCG) test (***if female***).

   NOTE: Pregnancy was also a criteria for discontinuation of any further dosing or non-safety related interventions for that subject.
2. Currently breast-feeding (***if female***).
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affected the ability of the participant to understand and comply with the study protocol.
4. Hemoglobin, WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal (subjects may have been included at the investigator's discretion for 'not clinically significant' values outside of normal range).
5. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal (subjects may have been included at the investigator's discretion for 'not clinically significant' values outside of normal range).
6. Infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B (HBV).

7. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies including urinalysis.
8. History of receiving any investigational product within the past 30 days.
9. Participation or planned participation in a clinical trial with an investigational product prior to completion of the follow up visit 28 days following last vaccination OR planned participation in an investigational vaccine study until the last required protocol visit
10. Subject has had medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
11. History of a severe allergic reaction or anaphylaxis.
12. Severe asthma, defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that required the use of oral or parenteral corticosteroids at any time during the past 2 years.
13. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia.
14. Known immunodeficiency syndrome.
15. Known asplenia or functional asplenia.
16. Use of chronic (≥14 days) oral or intravenous corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of Study Day 0.
17. Prior to Study Day 0 and every subsequent vaccination day, receipt of a live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks.
18. Receipt of immunoglobulins and/or blood products within the past 6 months.
19. Previous receipt of an investigational malaria vaccine in the last 5 years.
20. Other condition that in the opinion of the investigator would have jeopardized the safety or rights of a participant participating in the trial, interfered with the evaluation of the study objectives, or would have rendered the subject unable to comply with the protocol.
21. History of severe reaction to mosquito bites.
22. History of allergy to any component of the comparator vaccine (e.g. neomycin).

2.5 Study Objectives

2.5.1 Primary Objective

Primary objective of the study was to assess safety and reactogenicity of Pfs25M-EPA/Alhydrogel®, Pfs230D1M-EPA/Alhydrogel®, and simultaneous administration of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Malian adults

2.5.2 Secondary Objective

The secondary objectives of the study included determining the functional antibody response to the Pfs25 and Pfs230D1 protein as measured by ELISA and transmission blocking assays [Standard Membrane Feeding Assay (SMFA), and Direct Skin Feeding (DSF)] in Malian adults.

2.5.3 Exploratory Objective

Exploratory objectives included the following:

- To assess cellular and transcriptomic responses to the Pfs25 and Pfs230D1 vaccines when administered alone and in combination
- To evaluate the feasibility of using the "Experimental Hut" as a tool for vaccine efficacy measurement
- To evaluate the impact of co-infections on malaria vaccine responses
- To explore the antibody repertoire of functional antibody responses

2.6 Study Endpoints

2.6.1 Primary Endpoints

The primary endpoint of the study was the incidence of local and systemic adverse events and serious adverse events in Malian adults.

2.6.2 Secondary Endpoints

Secondary endpoints included the following:

- Anti-Pfs25 antibody levels elicited by Pfs25-EPA/Alhydrogel®, as measured by ELISA
- Anti-Pfs230D1 antibody levels elicited by Pfs230D1-EPA/Alhydrogel®, as measured by ELISA
- Transmission Blocking Activity (TBA) of induced antibody, as measured by SMFA
- TBA comparing Pfs25-EPA/Alhydrogel®, Pfs230D1-EPA/Alhydrogel®, simultaneous administration of Pfs25-EPA/Alhydrogel® and Pfs230D1-EPA/Alhydrogel®, and the comparator group, as measured by DSF

2.6.3 Exploratory Endpoints

Exploratory endpoints included the following:

- Cellular immune responses to vaccination
- Whole genome transcriptional profiling
- Antibody levels against recombinant EPA, and other malaria sexual stage antigens, such as Pfs48/45, expressed during the gametocyte development
- Experimental hut mosquito collections
- Schistosomiasis detection (in urine)
- qPCR for schistosomiasis and helminthes detection from stool
- Sequence B cell receptor/antibody genes

2.7 Symptomatic Malaria

In accordance with Malian national treatment guidelines, symptomatic malaria was defined as *Plasmodium* asexual parasitaemia accompanied by an axillary temperature of at least 37·5 °C and/or clinical signs and symptoms compatible with malaria. If a subject was diagnosed with symptomatic malaria, subjects were treated with artemether-lumefantrine. Doses were given orally, preferably with food, in accordance with the package insert and clinical practice. Asymptomatic parasitaemia was not treated.

Symptomatic malaria was reported as an AE. All malaria cases were reported as not related to vaccination. Asymptomatic parasitemia (positive blood smears without related malaria clinical symptoms) was not reported as an AE, but routinely captured during the course of the study.

2.8 Safety

2.8.1 Solicited Reactogenicity

All solicited (see **Table S1**) and unsolicited AEs were recorded through Day 14 after each vaccination. Injection site reactions (local reactogenicity) were assessed until Day 7 after vaccination or until resolved. Adverse reactions related to DSF were recorded until Day 7 after feeds or until resolved.

2.8.2 Laboratory Adverse Event

Protocol-specified laboratory assessments, including complete blood count with differential, creatinine (Cr) level, alanine aminotransferase level (ALT), and urinalysis (protein, blood) were completed prior to each vaccination and on days 7 and 14 after each vaccination. Local laboratory normal values were provided from the community for defining abnormal laboratory

values for the study (**Table S2**). Grading of abnormal laboratory values were completed as in **Table S3**.

Additional laboratory abnormalities other than those specified as safety labs in the protocol were also reported as AEs if they required intervention. Interventions included, but were not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality was reported as an AE at the discretion of the investigator. This included any laboratory results for which there was no intervention, but the abnormal value suggested a disease or organ toxicity.

2.8.3 Adverse Events Reporting

Unsolicited AEs (including symptomatic malaria), SAEs, unanticipated problems, and new onset of chronic illness were recorded throughout the vaccination period and transmission season. Symptomatic malaria episodes occurring during follow-up were recorded as a single diagnosis, not as individual symptoms, and was by default not related to vaccination.

2.9 Malaria Assessment

2.9.1 Blood Smears

Blood smears (BS) were prepared at specified time points during vaccination, paired with DSF, and when clinically indicated. Giemsa-stained thick and thin films were examined for asexual and sexual parasites in the MRTC clinical laboratory according to standard procedures. Subjects who were positive by blood smear but not fulfilling Mali National Policy on Malaria Control guidelines were followed for development of symptoms of malaria but were not offered antimalarial medications unless they developed symptomatic malaria.

We defined blood smear positivity as the detection of single *P. falciparum* asexual parasite per 1000 white blood cells. Gametocytemia was defined as ≥1 gametocyte seen per 1000 white blood cells. All gametocyte reads were confirmed by at least two certified microscopists. *P. ovale* and *P. malariae* asexual parasite infections were also captured and reported per 1000 white blood cells.

2.10 Immunogenicity

2.10.1 Enzyme Linked Immunosorbent Assay (ELISA)

Anti-Pfs25 and anti-Pfs230D1 ELISAs were performed on sera or plasma obtained from immunized subjects at the LMIV in Rockville, Maryland. Briefly, microwell plates were coated with antigen solution. Plates were washed with TRIS-buffered saline (TBS) containing Tween-20 (T-TBS) and blocked with TBS containing skim milk powder. After washing with T-TBS, diluted serum samples were added in triplicate and incubated at room temperature for 2 hours.

After incubation, unbound antibodies were removed by washing the plates with T-TBS, and alkaline phosphatase-conjugated goat anti-human IgG solution was added to each well and incubated for 2 hours at room temperature. Plates were then washed with T-TBS, followed by adding phosphatase substrate solution to each well; the plates were then covered and incubated for 20 minutes at room temperature for color development. The plates were read immediately at 405 nm with a microplate reader. The optical density values were used to calculate ELISA Units (EU) by comparing to a standard curve generated from a known positive-control plasma included on each ELISA plate. The limit-of-detection on a plate was derived from the standard curve.

2.11 Functional Activity

The transmission-blocking assays that were conducted are summarized below.

Transmission Blocking Assays

Assay	Mosquitoes	Test Samples	Site
Standard Membrane-Feeding Assay (SMFA)	Lab strain (*A. stephensi*)	Membrane feeds with lab cultured parasites mixed with test serum/plasma	LMIV
Direct Skin Feed (DSF)	MRTC lab colonies (*A. coluzzii*)	Direct skin feeds on vaccinees	MRTC
Experimental Huts (EH)	Wild-caught mosquitoes	Field mosquitoes trapped in a hut resided by a vaccinee	MRTC

Priority for feeding assays (SMFA and DSF) were conducted post Vaccination #3 and #4 when gametocyte carriage and parasite carriage rates were the highest and antibodies secondary to Pfs25M or Pfs230D1M were expected to be peaking post-vaccination. Multiple feeds were conducted on a single subject if parasites were present at the multiple screening time points, but a single subject did not undergo more than 12 DSF (inclusive of the twice a week feeds during the 6 DSF evaluation weeks following Vaccination #3 and #4) within any 12 month period while on study.

2.11.1 Standard Membrane Feeding Assay

SMFAs were performed on blood obtained at baseline and periodically after vaccination, with assays conducted at LMIV. In an SMFA, test serum or plasma obtained from immunized subjects was mixed with parasites from a laboratory culture and placed in a feeding cup covered with an artificial membrane. Pre-starved mosquitoes from a laboratory colony were allowed to

feed through the membrane. A similar procedure was carried out on a malaria-naïve control serum at the same time, using mosquitoes raised from the same laboratory colony. One week after the feed, mosquitoes were dissected and midguts were stained with mercurochrome and examined for the oocyst form of the parasite. The reduction of the proportion of oocyst-laden mosquitoes or the reduction of average oocyst numbers per mosquito, compared to mosquitoes fed on the control group, demonstrate biologic function of the antibody, and may be predictive of efficacy in the field. SMFA results have been shown to correlate with ELISA antibody titres against Pfs25 in several species.[3]

At the time of the DSFs, venous blood was also collected at defined timepoints (7, 14, 28, 42 days post dose 3 and 4) from each subject and processed immediately for SMFA. The process was maintained at approximately 37°C to avoid temperature-induced gametogenesis

2.11.2 Direct Skin Feeds

For DSFs, 2 feeding pints with at least 30 pre-starved female mosquitoes (post Vaccination #3) or at least 15 pre-starved female mosquitoes (post Vaccination #4) in each were prepared. Each subject was exposed to the feeding pints for 15-20 minutes. All subjects were offered a topical antihistamine and/or topical antipruritic to use following the feeds.

During the time periods outside of the intense twice-a-week DSF for 6 weeks following Vaccinations #3 and #4, attempts to identify and optimize parameters that contribute to DSF variability were implemented during the conduct of the feeding assays. Subjects were provided the details of the variation on the DSFs prior to participation, and were not required to deviate from the standard DSF (2 feeding pints; approximately 30 pre-starved female mosquitoes per pint; application to the bilateral calves or arms; exposure for 15-20 minutes; feed conducted at subject's convenience, usually dusk or dawn).

These variables included the following and results have been previously published[4]:

- Body location (for example, ankle, leg, forearm),
- Time of day feeds conducted (for example, dawn, dusk, late night),
- Number of feeds per day (up to 2 feeds in a single 24 hour period),
- Starvation time and age of mosquitoes used in the feeding assays

The total number of mosquitoes used for each DSF was maintained at approximately 60 (post Vaccination #3) and approximately 30 (post Vaccination #4) mosquitoes total regardless of these variables, and no subject underwent more than 12 DSFs (inclusive of the twice a week feeds during the 6 DSF evaluation weeks following Vaccination #3 and #4) within any 10 month period while on study.

After the feed, surviving mosquitoes were assessed for infectiousness by microscopy and/or molecular assays.

The following criteria were checked prior to each DSF and were contraindications to a DSF proceeding and may have resulted in delay of DSF or withdrawal from participating in further DSF:

- Severe local or systemic reaction to mosquito bites following a previous DSF
- Positive urine or serum β-hCG test (pregnancy testing results obtained within 7-10 days of the DSF are acceptable).
- Acute illness with an oral temperature >37·5˚C at the time of the DSF.
- Hemoglobin <8·5 g/dL (as seen on safety labs obtained following vaccination) or clinical concerns for anemia
- Any other condition that in the opinion of the Investigator poses a threat to the individual if immunized or that may complicate interpretation of the safety of vaccine following immunization.

Recent use of antimalarial medications was not a contraindication to participating in the DSF. The medication used and time period the medication was taken was recorded on the subject's source documents and case report forms accordingly.

2.11.2.1 Mosquito Rearing at the Insectary

A laboratory colony of *A. coluzzii* established from a local catch in 2008 was used for the DSFs. This colony had been used in the current assay development study, and was demonstrated to have similar susceptibility as the F1 progeny of the wild-caught mosquitoes. The colony had been maintained using blood meals collected under standard procedures of the blood transfusion center from local healthy donors. To ensure that the donor was free of potentially transovarial arbovirus in incubation, the donated blood was only used 5-7 days after the collection and after the donor was confirmed to remain healthy during this period.

The insectary in which mosquitoes were reared in Bamako had been in use for more than 15 years at the time of the study. Security was ensured by the use of double doors, which prevented the escape of reared mosquitoes as well as the entry of non-insectary mosquitoes. Mosquitoes were transported to Bancoumana in net-sealed feeding cups secured in wooden holders inside a cooler, with wet towels to maintain adequate humidity. After arrival at the assigned insectary in Bancoumana, mosquitoes were secured within the transporting containment. The insectary was adequately equipped with rooms with humidifiers which were regularly monitored according to

standard insectary procedures, and access to the insectary was limited to study personnel only. After feeds, mosquitoes, still in net-sealed cups, were transported back to the insectary in Bamako. All subsequent handling of mosquitoes took place in Bamako.

2.11.2.2 Dissections

Mosquitoes were knocked down by either freezing them or by agitating the pint without crushing them and then transferred into a Petri dish on ice containing a slightly wet paper towel. Under a dissecting scope and on a slide each individual specimen was placed in a drop of 0·5% mercurochrome solution. The midgut was pulled and covered with a coverslip. The oocysts were detected under a light microscope. The presence of oocyst and oocyst counts for each specimen were recorded. A mosquito was determined as a positive mosquito if ≥ 1 oocyst is present.

Select mosquito heads and thoraces were processed for ELISA to detect sporozoites and for PCR to identify species and molecular form of the mosquito.

2.12 Exploratory Studies

2.12.1 Experimental Huts

2.12.1.1 Experimental Huts and Volunteers

To evaluate the feasibility of using the "Experimental Hut (EH)" as a tool for vaccine efficacy measurement, adults who were parasite positive (asexual and/or sexual stages) and participated in mosquito feeding assays were invited to participate in EH studies conducted the night after a DSF assay. EH were set up whereby the individual was asked to sleep alone overnight in their own hut which was modified such that windows, door and the eaves of the rooms were sealed and an exit traps were installed at one window exit. The participant was invited to sleep without changing any of their regular behavior (e.g. sleep time, bed net etc.) excepting that other family members were required to sleep elsewhere for that night.

2.12.1.2 Mosquito collection

Live wild mosquitoes were collected via exit traps installed on an open window of the dwelling which was installed from approximately 6PM to 6AM the next morning. Following exit trap collection, pyrethrum spray catches were conducted in the room. Mosquitoes were morphologically identified to species (*Anopheles gambiae s.l.*) and their blood feeding status was determined (fed vs unfed). Mosquito bloodmeals were dissected out and individually preserved in Qiagen Buffer AL while the remainder of mosquito carcasses were individually preserved in buffer RLT to preserve DNA/RNA for genotyping experiments.

2.12.1.3 Extraction of Nucleic Acids and Forensic Typing

Bloodspots were collected from study participants on Whatman 903 Savercards (GE Healthcare). DNA/RNA was extracted (Qiagen Allprep Mini) and integrity of DNA extracted from mosquito bloodmeal was confirmed through 28s PCR and Human b-actin qPCR to confirm presence of both human and mosquito genetic material. Blood samples (either from mosquito bloodmeal or reference sample from study participant) were genotyped using a commercially available forensic fingerprinting kit (Powerplex 16, Promega). Samples were electrophoresed on an ABI 3730xL capillary array and analyzed with Genemapper 3.2 (ABI) sequence analysis software to generate electropherograms based on Local Southern sizing based off of the included ILS600 Ladder; allelic peaks and genotypes were assessed within a margin of +/- 1 base in reference to the Powerplex Allelic Ladder. To reduce the probability of human bias and raise the effort to forensic standards, the use of Genemarker HID (Promega/Softgen) CODIS-certified forensic typing software to generate profiles from the bloodmeals in mosquitoes and compared to profiles generated from bloodspots of human hut participants was incorporated. Profiles from bloodmeals that matched the EH participant were called Internal Feeds. Profiles from mosquitoes that were collected in EHs that did not match the profiles generated from bloodspots of the hut participant were labeled External Feeds. Mosquito bloodmeal profiles that showed evidence of feeding on multiple individuals were called Multiple Feeds and were divided into Internal Multiple Feeds (where a mosquito did feed on an individual inside the hut and another outside) and External Multiple Feeds (where a mosquito exhibited evidence of multiple feeds, but neither profile in the bloodmeal matched the EH study participant). In cases where DNA extracted from EH mosquitoes did not generate a profile by the forensic typing kit, presence of human blood in the mosquito was confirmed by using a human b-actin qPCR probe.

2.12.2 Co-infections

A single stool and urine sample was collected from willing subjects at screening. Stool samples were aliquoted and cryopreserved at -80°C in Mali and then shipped to the U.S. on dry ice for analysis via a modified qPCR as previously described.[5,6] *Schistosoma haematobium* eggs were quantified in real time by microscopy after filtration of fresh urine samples. Individuals diagnosed with urinary schistosomiasis were treated with praziquantel.

2.12.3 DSF Midgut Plasmodium Species Identification

We constructed a speciation library of 26 distinct ribosomal 18S reference DNA sequences from 12 plasmodium species, including all expected human malaria parasites and several rodent parasites added as negative controls (mean=2·2 constructs per species, min=1, max=6). All reference DNA sequences were truncated to match the region of 18S targeted by the PCR amplification primers.

Mosquito midguts were collected in ~200 mL of Qiagen RLT Plus Buffer at the time of dissection and oocyst confirmation and stored at -80°C prior to DNA extraction. Genomic DNA

was extracted from midguts using a AllPrep® DNA/RNA Micro Kit (Qiagen, UK) following the manufacturer's suggested protocol for gDNA recovery with a few modifications. Midgut samples were thawed on ice and then homogenized using individual, disposable, pre-sterilized pestles. 200 mL of RLT Plus buffer was added to the homogenized sample and the sample then vortexed for approximately 15 seconds. The lysates were centrifuged at max speed (≥ 14,000 rpm) for 3 minutes. The supernatant from each sample was transferred to an AllPrep DNA spin column and centrifuged at 8,000 g for 30 seconds and the flow through discarded. 500 mL of Buffer AW1 was added to the column. The samples were centrifuged at 8,000 g for 30 seconds and the flow through discarded. 500 mL of Buffer AW2 was added to the column. The samples were centrifuged at max speed (≥ 14,000 rpm) for 2 minutes and the flow through discarded. The samples were again centrifuged at max speed (≥ 14,000 rpm) for 1 minute to ensure no Buffer AW2 carryover. The AllPrep DNA column was placed in a new, pre-sterilized 1·5 mL collection tube and 40 mL (pre-heated to 70°C) Buffer EB was added to the center of the column membrane. Samples were incubated at room temperature for 3 minutes and then centrifuged at 8,000g for 1 minute. The eluate was re-loaded onto the column and incubated at room temperature for another 3 minutes and then again centrifuged at 8,000g for 1 minute. Resulting gDNA was kept on ice until use in PCR assay(s). All midgut gDNA was used in PCR assays on the same calendar day (usually within 2-3 hours of extraction) without any freeze/thaw cycles.

The Applied Biosystems 7500 Fast and QuantStudio5 Real-Time PCR systems were used for qRT-PCR amplification in the Pan-Plasmodium 18S '*Genus'* assay. 5 mL of gDNA was used in each 25 mL PCR reaction. Samples were run in triplicate. QuantiTect SYBR Green RT-PCR Master Mix (Qiagen, UK) was used with Pan-Plasmodium primers (*Genus* 18S Forw, 5′-TAACGAACGAGATCTTAA -3′; *Genus* 18S Rev, 5′- GTTCCTCTAAGAAGC**W**TT-3) at final a concentration of 900 nM. The reverse primer contains a single degenerate base to cover a broad spectrum of Plasmodium species. After an initial PCR activation step (95°C for 15 minutes) conditions were as follows: denaturation at 95°C for 5 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 30 seconds for 40 cycles. Resulting amplified product from the triplicates was pooled and purified, using a QIAQuick PCR Purification Kit (Qiagen, UK), prior to being submitted for sequencing.

The PCR product for each midgut was Sanger sequenced by Eurofins using separate forward and reverse sequencing primers to generate 2 DNA base call sequences and 2 raw ABI chromatogram files per midgut.

Each midgut DNA base call sequence was scored against the reference library using the pairwiseAlignment() function from R package Biostrings, and selects the reference sequence having the highest alignment score as the called species. A p-value for the call was generated using a 1-sample T test comparing the top score against the scores for the 3 next best-scoring

species. To be considered a valid species call, the best score must have been at least 50% of a perfect match score (to exclude truncated or corrupted base call sequences) and have a p-value below 0·05. Otherwise, the algorithm returned a FAIL call, since the DNA base call sequence from the chromatogram did not unambiguously select one and only one species.

2.13 Statistical Analysis

2.13.1 Antibody Decay Model Formulation

We fit the following model separately for the Pfs230D1 and Pfs25 antigens. Let j index the number of doses received and k index the plate on which a sample was run. The operational time scale, t, is time in years since antibody titres were assumed to last peak, corresponding to the time-point two weeks after the most recent dosing event. Note that operational time resets after each dose administration. We denote the log titre at time t for subject i having received j doses measured on assay plate k by $Y_{ijk}(t)$. Let $\boldsymbol{\theta}$ denote the vector containing all model parameters and having prior $\pi(\boldsymbol{\theta})$. Let $1\{t_i\}$ be a treatment indicator for participant i taking value 1 if the vaccines was administered in combination and 0 otherwise, and let $1\{d_j\}$ be an indicator taking value 1 if exactly j doses have been administered and $t > 14$ days, such that titres have peaked, and 0 otherwise. Finally, let b_i denote a participant-level random effect, $c_i j$ denote the participant random effect at dose j, and p_k denote a plate random effect. The statistical model is formulated as

$$
\begin{aligned}
Y_{ijk}(t) &\sim N(\mu_{ijk}(t), \sigma_{ij}(t)^2),\\
\mu_{ijk}(t) &= \log(C_{ijk}(0)) + \tilde{\lambda}_i t^{\kappa_i},\\
\log(C_{ijk}(0)) &= \beta_0 + \sum_{j=1}^{4} 1\{d_j\}(\beta_j + \delta_j * 1\{t_i\}) + b_i + c_{ij} + p_k,\\
\log(\tilde{\lambda}_i) &= \eta_0 + \eta_1 1\{t_i\},\\
\mathrm{logit}(\kappa_i) &= \gamma_0 + \gamma_1 1\{t_i\},\\
\log(\sigma_{ij}) &= \phi_0 + \phi_1 \log(1+t) + \sum_{j=2}^{4} 1\{d_j\}\phi_j \log(1+t)\\
b_i &\sim N(0, \tau^2),\\
d_{ij} &\sim N(0, \psi^2),\\
p_k &\sim N(0, \xi^2),\\
\boldsymbol{\theta} &\sim \pi(\theta).
\end{aligned}
$$

Let δ_{ijk} be an indicator for whether Y_{ijk} is above the LoD for plate k taking a value of 1 if $Y_{ijk} < LoD_k$ and 0 otherwise. Observations above the LoD, i.e., $\delta_{ijk} = 0$ make Gaussian density contributions to the likelihood. To account for censoring, we could treat censored observations as parameters in the model and sample them explicitly in each Markov Chain Monte Carlo (MCMC) iteration by drawing $Y_{ijk}^{\delta_{ijk}=1} \sim N\left(\mu_{ijk}(t), \sigma_{ij}(t)^2\right)$. However, it is more efficient to integrate out the missing observations. Hence, each censored observation contributes a Gaussian CDF term to the likelihood. Hence, the likelihood is

$$
L(\mathbf{Y} \mid \theta) = \prod_i \prod_j \left[\phi\left(Y_{ijk}; \mu_{ijk}(t), \sigma_{ij}(t)^2\right)^{1-\delta_{ijk}} + \Phi\left(LoD_k; \mu_{ijk}(t), \sigma_{ij}(t)^2\right)^{\delta_{ijk}}\right],
$$

where $\phi\left(\mu_{ijk}(t),\ \sigma_{ij}(t)^2\right)$ and $\Phi\left(\mu_{ijk}(t),\ \sigma_{ij}(t)^2\right)$ are Gausian probability density and cumulative density functions, respectively, and LoD is the assay limit of detection.

We used the following priors, which are weakly informative on the scale of the data, in specifying our model:

We assessed the sensitivity of our inferences to more and less diffuse choices of priors replacing $\mathrm{Normal}(0, 2{\cdot}5^2)$ priors with $\mathrm{Normal}(0,1)$ and $\mathrm{Normal}(0,10)$ distributions, and replacing $\mathrm{Exponential}(1)$ distributions with $\mathrm{Exponential}(\mathrm{mean} = 10)$ priors and found no discernable differences. Posterior samples were drawn using the No U-Turn variant of Hamiltonian Monte Carlo implemented in Stan, and the model was implemented using brms. We ran four MCMC chains for 2,000 iterations each, discarding the first 1,000 iterations of each chain as warmup,

694 and combining the remaining samples from all chains. Convergence of MCMC was assessed
695 visually and by verifying that all potential scale reduction factors were less than 1.

696 To check whether hypothetical data simulated from the model resembled trial data, we examined
697 antibody decay model predictive distribution versus observed distribution with crude imputation
698 for samples below the limit of detection.

699 ### 2.13.2 Comparison of Antibody Decay Model Predictions with Crude LoD Imputation

700 The number of doses needed for each participant[7] to elicit an immune response was defined in
701 this supplementary analysis by counting the number of doses until the peak antibody titre
702 exceeded a pre-defined threshold that was set based on baseline titres in the TWINRIX/Menactra
703 + NS comparator arm. Peak antibody response was assumed to occur at each two-week post-dose
704 timepoint. The thresholds for declaring an immune response for Pfs25 and Pfs230D1 were
705 additionally adjusted for batch effects in the limit of detection and, in the case of Pfs25, evidence
706 of prior infection (details in **Section 4.2)**.

707 Comparison of the model posterior predictive distributions with crude pointwise summaries of
708 the data indicated the model was concordant with key features of the data. Specifically, the
709 pointwise posterior predictive mean values and decay profiles were in strong agreement with
710 those calculated from the raw data with the samples below the plate LoDs imputed at half the
711 limit of detection. Quantiles of the posterior prediction intervals and crude 95% density intervals,
712 as expected, differed due to handling of censored data.

713 ### 2.13.3 Mali versus U.S. study

714 In a post-hoc analysis, TBV antibody responses in this Malian population were compared to
715 those in the preceding U.S. cohort (N=5/arm) that received two doses under the same protocol.[1]
716 Vaccinations were administered on a 0, 1 month schedule at the same doses ("low dose": Pfs25 =
717 16µg, Pfs230D1 = 15µg; "high dose": Pfs25 = 47µg, Pfs230D1 = 40µg) for all subjects. Malian
718 participants in the main phase received three doses on a 0, 1, 4·5, 16·5 month schedule, but only
719 antibody responses through 3 months post dose 2 (prior to receipt of dose 3) were analysed for
720 comparison.

721 # 3 RESULTS

722 ## 3.1 Safety

723 ### 3.1.1 Pilot Safety Cohort Results

724 Vaccinations in the low dose, pilot safety arms (16 µg Pfs25 alone; 15 µg Pfs230D1 alone; 16 µg
725 Pfs25 + 15 µg Pfs230) versus comparator (TWINRIX +/- NS) were relatively well-tolerated as
726 described in the main text. Local and systemic reactogenicity are presented in **Table S5**, and

laboratory abnormalities are presented in **Table S6**. Summary of safety data for the pilot safety cohort reported during the study can be found in **Table S7**.

3.1.1.1 16 µg Pfs25 alone

All AEs (19/19) reported in the 16 µg Pfs25 alone arm were mild or moderate (Grade 1 or 2) (**Table S7)**. The only reported related AEs were injection site pain (all Grade 1), which did not increase in frequency with subsequent vaccination (**Table S5**). No solicited systemic AEs were reported (**Table S5**) and only one laboratory abnormality (Grade 1, thrombocytopenia) was noted **Table S6**). No Grade 3 or 4 AEs were reported; no SAEs were reported (**Table S7)**.

3.1.1.2 15 µg Pfs230D1 alone

For 15 µg Pfs230D1 alone arm the majority of reported AEs (14/17) were also mild or moderate (Grade 1 or 2) (**Table S7)**. No local reactogenicity was reported post-dose 1 or dose 2 (**Table S5**). Only one solicited systemic AE (Grade 1 headache) was reported (**Table S5**). Except for the laboratory abnormalities as detailed below, only two laboratory AEs (both Grade 1, leukocytosis and leukopenia) were reported post-dose 2 (**Table S6**). No SAEs were reported (**Table S7)**.

Post-vaccination 1, one subject (31-year-old male) experienced an acute onset of Grade 3 gastroenteritis (presented with headache, myalgias, vomiting, diarrhea) with associated Grade 4 laboratory abnormalities (leukocytosis = white blood cell (WBC) count was $26{\cdot}1x10^3$/µl, blood creatinine increased = 244·91 µmol/l (2·8 mg/dL), noted to be Grade 4, though not requiring dialysis). At that time, based on clinical exam and laboratory results, acute gastroenteritis with dehydration was diagnosed by the clinician. Subject was prescribed treatment with antibiotics (ciprofloxacin and metronidazole) during that visit with plan for close follow up. A follow up visit was completed two days later, at which time the subject's recent medical history and clinical evaluation revealed continued symptoms (vomiting, nausea, dizziness) with worsening signs of dehydration (dry mouth, low blood pressure 100/60 mmHg of blood pressure). An IV was placed and intravenous rehydration was provided (Lactated Ringer's, 5% glucose) with noted improvement in hydration status following receipt of fluids. Due to continued symptoms and clinical appearance, the subject was given ceftriaxone and metoclopramide and continued on antibiotics previously prescribed. The subject was seen the following two days at the clinic with noted resolution of signs/symptoms of dehydration but continued, but improved, abdominal pain, diarrhea, and vomiting. His laboratory abnormalities were repeated five days from his initial labs and it was noted that his WBC and absolute neutrophil count had normalized and his creatinine had improved to 165·22 µmol/l (1·9 mg/dL); labs repeated 7 days later showed his WBC and absolute neutrophil count were again normal, while his creatinine value continued to improve to 119·29 µmol/l (1·3 mg/dL). However, due to persistence of symptoms, the subject was referred to an outpatient internal medicine specialist for further evaluation with subsequent diagnosis confirmed as acute gastroenteritis from the clinical provider; this subject was deferred from

proceeding to vaccination 2 given ongoing clinical work-up and continued Grade 1 laboratory abnormalities.

3.1.1.3 16 µg Pfs25 + 15 µg Pfs230D1

For the 16 µg Pfs25 + 15 µg Pfs230D1 co-administration arm, the majority of AEs (24/25) were mild or moderate (Grade 1 or 2) (**Table S7)**. As expected, given two investigational vaccines were co-administered in two separate extremities, local reactogenicity and related AEs commonly appeared in both rather than one arm, reported for the co-administered combination group compared to single antigen pilot safety arms, in particular when compared to Pfs230D1 alone arm (**Table S5, S7**). Only one solicited AE (Grade 1, headache) was reported (**Table S5)**. One individual experienced Grade 1 neutropenia post each vaccination (**Table S6**). No Grade 4 or SAEs were reported. One subject experienced an unrelated Grade 3 AE (malaria, not related, unsolicited AE, starting ~3·5 months post-last vaccination) (**Table S7)**.

3.1.1.4 TWINRIX +/- NS

Subjects receiving either TWINRIX alone (N=5) or TWINRIX + normal saline (NS, N=5) were combined for analysis purposes given recruitment from a similar population/community, close proximity of enrolment, and same follow-up per protocol. Overall, subjects receiving the comparator vaccine experience mainly (29/30) mild or moderate (Grade 1 or 2) (**Table S7**). Local reactogenicity was infrequently reported and when accounting for vaccine receipt by arm, overall was most often reported post-dose 2 and accountable to TWINRIX, not normal saline (**Table S5**). No solicited AEs were reported (**Table S5**). One individual experienced Grade 1 thrombocytopenia post each vaccination (**Table S6)**. No Grade 4 or SAEs were reported (**Table S7)**. One subject experienced an unrelated Grade 3 AE (nasopharyngitis, not related, unsolicited AE, starting ~4 months post last vaccination) (**Table S7)**.

3.1.1.5 Serious Adverse Events

No serious adverse events were reported during the pilot safety cohort.

3.1.1.6 Symptomatic Malaria

Symptomatic malaria AEs post-dose 2 in pilot phase were all grade 1/2 (Pfs25: 1, Pfs230: 2, Pfs25+Pfs230: 6, comparator: 5). As expected, malaria AE increased in reporting as the study entered into the malaria transmission season with 85·7% (12/14) cases being reported between August to November 2015. Average parasitemia (parasites/1000 WBC) associated with symptomatic malaria was not significantly different between arms (Pfs25: 900, Pfs230: 1149, Pfs25 + Pfs230: 566, comparator: 1802) but arm sizes were not powered for this endpoint.

3.1.2 Main Cohort Results

Vaccinations in the high dose, main cohort arms (47 µg Pfs25 + NS; 40 µg Pfs230D1 + NS; 47 µg Pfs25 + 40 µg Pfs230) versus comparator (TWINRIX, Menactra +/- NS) were relatively well-tolerated as described in the main text. Local and systemic reactogenicity are presented in **Table S8-9**, and laboratory abnormalities are presented in **Table S10**. Summary of safety data for the main cohort reported during the study can be found in **Table 2**.

Most commonly reported AEs were Grade 1/2 (**Table 2**), and most related AEs in Pfs25 and Pfs230D1 arms were injection site reactogenicity, reported more frequently for Pfs25 and Pfs230D1 arms than comparator.

Attribution of local reactogenicity was much more common in the Pfs25 or Pfs230D1 administered arms rather than normal saline as well as with comparator vaccine for dose 2, 4 (**Table S8).** Similar frequency of local reactogenicity in the Pfs25+Pfs230D1 arm was attributed to either Pfs25 or Pfs230D1 **(Table S8).** Local AEs did not increase in frequency with successive doses of Pfs25 and Pfs230D1, but there was an increase in severity (higher Grade 2 frequency) of local injection site pain seen with Pfs25-based regimens **(Table S8;** in the Pfs25 alone arm: dose 2 vs dose 4, p=0·009; for the combination arm: dose 1 vs dose 2, p=0·0072, dose 3 vs dose 4, p=0·0127 **)**.

Solicited AEs were few in all arms with headache being the most common; Pfs25+Pfs230D1 did see an increase in solicited AEs post-dose 4 (p=0·0387; **Table S9)**. Reporting of laboratory abnormalities post vaccination were similar across all arms **(Table S10)** with all being Grade 1/2 except for two subjects (1 Pfs230D1 + NS, 1 TWINRIX + NS) reporting two unrelated Grade 4 laboratory abnormalities (both blood creatinine increased).

3.1.2.1 47 µg Pfs25 + NS

Safety analysis of 47 µg Pfs25 + NS showed the majority of AEs reported during the course of the study were either Grade 1 (227/632, 35·9%) or Grade 2 (393/632, 62·2%) (**Table 2**); majority of participants reported at least 1 AE during the trial (98%).

Local reactogenicity was common (42/50 subjects, 84%) and was most frequently attributed to Pfs25 vaccinated arm at all dosing timepoints (**Table S8**). Injection site pain was the most commonly reported local site reaction. Frequency and severity of local reactions attributed to Pfs25 were highest at post dose 2 and 4 **(Table S8**). No Grade 3 or higher local reactogenicity was reported.

Solicited reactogenicity was not commonly reported (<10% at any vaccination time point) and all AEs reported were Grade 1 or 2 (**Table S9**). Headache was the most commonly reported solicited AE at all vaccination timepoints (**Table S9**), though reported at a similar frequency as comparator.

Laboratory abnormalities were similar to the comparator arm at all vaccine doses, except post dose 4 Pfs25 + NS no participants developed laboratory abnormalities post vaccination (**Table S10**). All laboratory AEs reported were Grade 1 or 2 and were a variety laboratory abnormalities (**Table S10**). No significant laboratory trends were seen.

No SAEs were reported. In total, 12 Grade 3 AEs (12/632, 1·9%; all unrelated to vaccination) were reported during the course of the study. No Grade 4 or 5 AEs reported (**Table 2**).

3.1.2.2 40 µg Pfs230D1 + NS

Safety analysis of 40 µg Pfs230D1 + NS showed the majority of AEs reported during the course of the study were either Grade 1 (204/513, 39·8%) or Grade 2 (299/513, 58·3%) (**Table 2**); majority of participants reported at least 1 AE during the trial (98%).

Local reactogenicity was common (38/49 subjects, 77·6%) and was most frequently attributed to Pfs230D1 vaccinated arm at all dosing timepoints (**Table S8**). Injection site pain was the most commonly reported local site reaction. Frequency and severity of local reaction did not change significantly with subsequent vaccinations **(Table S8**). Grade 2 local reactions were infrequently reported throughout each dose.

Solicited reactogenicity were few and all AEs reported were Grade 1 or 2 (**Table S9**). Headache was the most commonly reported solicited AE at all vaccination timepoints except dose 3 when only arthralgia was reported by one participant (**Table S9**). No significant trends in solicited reactogenicity was seen when compared to comparator arm.

Laboratory abnormalities were similar to the comparator arm at all vaccine doses (**Table S10**). All laboratory AEs reported were Grade 1 or 2 except for a Grade 4 blood creatinine increased seen post dose 3 in a single subject that was determined unlikely related to vaccination given preceding history (**Table S10**). Overall, no significant laboratory trends were seen.

Two SAEs were reported in Pfs230D1 + NS (snake bite, peritonsillar abscess; summarized below in **Section 3.1.2.5; Table 2**) and were determined unrelated to vaccination prior to unblinding. In total, nine Grade 3 AEs (9/513, 1·8%; all unrelated to vaccination) were reported during the course of the study; one Grade 4 (blood creatinine increased as noted above). No Grade 5 AEs reported (**Table 2**).

3.1.2.3 47 µg Pfs25 + 40 µg Pfs230D1

Safety analysis of 47 µg Pfs25 + 40 µg Pfs230D1 showed the majority of AEs reported during the course of the study were either Grade 1 (287/668, 43%) or Grade 2 (373/668, 55·8%) (**Table 2**); majority of participants reported at least 1 AE during the trial (98%).

Local reactogenicity was common (40/50 subjects, 80%) and was equally attributed to Pfs25 or Pfs230D1 at each vaccination (**Table S8**). As expected, with each arm receiving either Pfs25 or Pfs230, double the number of local reactogenicity AEs were reported at each vaccination, but the overall frequency of participants complaining of local site reactions was unchanged compared to Pfs25 + NS or Pfs230D1 + NS (**Table S8**). Injection site pain was the most commonly reported local site reaction. Frequency did not change significantly with subsequent vaccinations, but severity of local reactogenicity increased with dose 4 (**Table S8**).

Solicited reactogenicity were infrequently reported except post dose 4 in the combination arm where 8 events were observed (p=0·0143); all AEs reported were Grade 1 or 2 (**Table S9**). Headache was the most commonly reported solicited AE at all vaccination timepoints (**Table S9**).

Laboratory abnormalities were similar to the comparator arm at all vaccine doses (**Table S10**). All laboratory AEs reported were Grade 1 or 2 and were a variety laboratory abnormalities (**Table S10**). No significant laboratory trends were seen.

One SAE was reported in Pfs25 + Pfs230D1 (cerebrovascular accident (CVA); summarized below in **Section 3.1.2.5; Table 2)**. Given the resultant death, this SAE was reviewed by Sponsor, Institutional Review Board (IRB), Faculté de Médecine Pharmacie d'OdontoStomatologie (FMPOS) Ethics Committee (EC), DSMB, and U.S. Food and Drug Administration (FDA) and prior to unblinding was determined unrelated to the vaccine.

In total, 7 Grade 3 AEs (7/668, 1%; all unrelated to vaccination) were reported during the course of the study. No Grade 4 AEs, and one Grade 5 AE (death) as previously described.

3.1.2.4 TWINRIX/Menactra + NS

Safety analysis of the comparator arm showed the majority of AEs reported during the course of the study were either Grade 1 (190/525, 36·2%) or Grade 2 (325/525, 61·9%) (**Table 2**); majority of participants reported at least 1 AE during the trial (98%).

Local reactogenicity was common (21/51 subjects, 41·2%) but reported significantly less in the comparator arm than Pfs25+NS (84%), Pfs230D1+ NS (77·6%), or Pfs25+Pfs230D1 arms (80%). Injection site pain was the most commonly reported local site reaction. Reporting of local site reactions related to the comparator vaccine (TWINRIX or Menactra) versus normal saline was similar for dose 1 and 3, but there was a notable increase in reported local site reactions post dose 2 of TWINRIX and with receipt of Menactra at dose 4 (**Table S8**). All local site reactions post receipt of TWINRIX were Grade 1 while Menactra local site reactions were equally reported as Grade 1 or 2 (**Table S8**). Both local reactogenicity safety profiles of TWINRIX or Menactra were consistent with prior reports.

Solicited reactogenicity was not commonly reported (<10% at any vaccination time point) and all AEs reported were Grade 1 or 2 (**Table S9**). Headache was the most commonly reported solicited AE at all vaccination timepoints (**Table S9**).

Laboratory abnormalities were similar at all vaccine doses (**Table S10**). All laboratory AEs reported were Grade 1 or 2 except for a Grade 4 blood creatinine increased seen post dose 1 in a single subject that was determined unlikely related to vaccination given preceding history but did not receive further vaccinations and was followed for safety (**Table S10**). Overall, no significant laboratory trends were seen.

No SAEs were reported. In total, 9 Grade 3 AEs (9/525, 1·7%; all unrelated to vaccination) were reported during the course of the study; one Grade 4 (blood creatinine increased as noted above). No Grade 5 AEs reported (**Table 2**).

3.1.2.5 Serious Adverse Events

During the study period, 3 SAEs were reported in the main cohort as summarized below. All were determined unrelated to vaccination. All of these subjects completed 4 vaccinations. No participants were removed from study participation due to a related AE of any severity.

40 µg Pfs230D1M-EPA/Alhydrogel AND normal saline

Snake bite (unrelated; hospitalization) – 48-year-old male bitten by a snake. He was admitted to Point G Hospital in Bamako, Mali due to abnormal coagulation and received anti-venom, analgesic, and antibiotics. Resolved without complication or sequelae.

Peritonsillar abscess (unrelated; hospitalization) – 45-year-old male with acute onset of fever, headache, odynophagia, and subsequent development of a peritonsillar abscess treated with IV antibiotics as well as incision and drainage, and resulting in hospitalization. Resolved without complication or sequelae.

47 µg Pfs25M-EPA/Alhydrogel AND 40 µg Pfs230D1M-EPA/Alhydrogel

Cerebrovascular accident (CVA; unrelated; death) – 51-year-old female with no significant past medical history presented with acute onset of altered consciousness and left hemiplegia and subsequently admitted to the hospital for further evaluation. CT scan completed and confirmed CVA with associated mass effect on the ipsilateral ventricles. Overnight she developed severe hypertension, respiratory distress, right hemiplegia, and seizures despite medical management and died a day after presentation.

3.1.2.6 Symptomatic Malaria

For fair comparison between study year 1 (2015-2016) and year 2 (2016-2017), symptomatic malaria cases reported were assessed for a 6-month period post dose 3 and dose 4. Post dose 3, from September 2015 to February 2016, 117 cases symptomatic malaria were reported (Pfs25: 31, Pfs230: 22, Pfs25+Pfs230: 31, comparator: 33) and as previously reported, symptomatic malaria cases in adult Malians was fairly common with 63·6% of comparator subjects reported at least 1 symptomatic malaria AE (**Table 2)**. Symptomatic malaria events least occurred in the Pfs230D1 alone arm (22 symptomatic malaria AEs, duration: 5 days, average parasitemia: 332 parasites/1000 WBC). Symptomatic malaria events were similar in Pfs25 and Pfs25+Pfs230D1 arms (n=31/arm; average parasitemia Pfs25: 541 parasites/1000 WBC, Pfs25+Pfs230D1: 460 parasites/1000 WBC). No significant differences in the magnitude of parasitemia were observed between arms. Comparing unique individuals by arm, the Pfs230D1 arm had marginally less symptomatic malaria AEs than comparator (p= 0·07); no other significant differences were noted between arms.

In the 6-month period following the booster dose (September 2016 until March 2017), similar trends were seen as had been noted in year 1. More symptomatic malaria events were observed in the Pfs25 arm (Pfs25: 31, Pfs230: 25, Pfs25+Pfs230: 26, comparator: 26) (**Table 2)**. Mean parasitemia associated with symptomatic malaria were also higher in both Pfs25 arms, but were not statistically different from the Pfs230D1 alone or comparator.

3.2 Pregnancies

Females of childbearing potential were enrolled and per inclusion criteria were required to use reliable contraception from 21 days prior to vaccination #1 to 3 months after the last vaccination. During the course of the study, one woman in the main cohort, who was appropriately on protocol specified pregnancy prevention (depot medroxyprogesterone), was noted to have a positive pregnancy test (urine, blood;10 June 2015) prior to her scheduled second vaccination (28 days post receipt of vaccination #1). The subject reported she had menstrual bleeding that started 2 days prior to her positive pregnancy test. She was deferred from receipt of vaccination and at that time the study team attempted to schedule her for an OB/GYN visit at Bamako Health Center but she refused.

Per request from the principal investigators, clinical Sponsor, and Medical Monitors, it was requested she undergo intentional, unscheduled unblinding to provide appropriate counseling for her pregnancy. She was identified to have received TWINRIX + NS for dose 1.

Initial follow-up with the women was complicated by refusal to return to clinic for safety follow-up, but it was determined approximately a year later (March 2016) that she continued to have her menstrual cycle as scheduled post coming off the study. Considering the urine and blood pregnancy test results were positive on 10 June 2015 and reported history of menstrual bleeding

on 08 June 2015 and no progression to pregnancy or further intervention, a spontaneous miscarriage is the final determination and outcome.

3.3 Major Protocol Deviations

During the course of the study three serious protocol deviations were reported.

- Vaccine administration error (May 2015) – Subject received a non-indicated vaccine by error from the pharmacy. One subject randomized to Pfs230D1, 40µg + normal saline was erroneously administered comparator for vaccination #1; reviewed by study team, statistician, Sponsor, and DSMB and recommended the subject continue to receive comparator for the rest of the study (subject and clinical team remained blinded); for analysis considered comparator subject (for as-treated analysis) and Pfs230D1 subject (for ITT).
- Vaccine administration error (June 2015) –Two subjects, both Malinke males from Koursale with the same name (first and last name), arrived for Study Day 28 (Vaccination #2) and the first participant was misidentified by the site investigator as the other subject resulting in one subject being administered Pfs25, 47µg + Pfs230D1, 40µg instead of Pfs230D1, 40µg + NS for vaccination #2 (received Pfs230D1, 40µg + normal saline for vaccination #1, #3; received Pfs25, 47µg + Pfs230D1, 40µg for vaccination #2); considered Pfs230D1 subject for both as-treated and ITT analysis.
- Laboratory error (October 2015) – Per time documentation by the CAP lab, a DSF was performed before one subject's blood and urine samples were collected and resulted (normal hemoglobin, negative pregnancy test) to determine subject ‘s hemoglobin and pregnancy status prior to undergoing DSF. Both tests were necessary to be confirmed prior to final determination of DSF eligibility.

3.4 ELISA

3.4.1 Antibody responses by gender

Antibody titres as measured by OD ELISA units against Pfs230D1 and Pfs25 were stratified by females and males at 2 weeks post-dose 3 and post-dose 4. Among females and males, median anti-Pfs230 antibody titres at 2 weeks post-dose 3 were 107 vs. 51 (Range 16-2022, 13-1055); at 2 weeks post-dose 4: 199 vs. 161 (15-5277, 15-1382), respectively. Median anti-Pfs25 antibody titres at 2 weeks post-dose 3 were 133 vs. 75 (16-1194, 16-995); at 2 weeks post-dose 4: 194 vs. 171 (15-2325, 15-3505), respectively.

3.4.2 Anti-EPA

Antibody levels against EPA were detected in each vaccinated group after the first dose, and peak titres increased after each dose (**Figure S6**). Vaccinated groups did not significantly differ in anti-EPA antibody levels 2 weeks post-each vaccination dose, except for the following:

Pfs230D1 single antigen vs. the combination arm at 2 weeks post-dose 3 (p =0·0057) and post-dose 4 (p<0·0001); and Pfs230D1 vs Pfs25 single antigens at 2 weeks post-dose 4 (p=0·0014).

3.5 Immune Response Modelling

The cutoffs for declaring an immune response for Pfs230D1 and Pfs25, respectively, corresponded to increases of 0·21 and 0·041 $\log_{10}$ ELISA units relative to the batch limit of detection (LoD). The effect of vaccine arm on the number of doses needed to elicit an immune response was assessed via a Bayesian proportional odds logistic regression model. Details regarding model specification and the model fitting procedure are provided in the Methods Section of the SA.

Based on the model, the majority of participants were expected to have titres to Pfs230D1 or Pfs25 by the time they receive 2 doses of their assigned treatment. Administering Pfs230D1 and Pfs25 in combination did not affect the expected number of doses needed to elicit a Pfs230D1 response (COR, 1·08; 95% CI, 0·52, 2·21) or a Pfs25 response (COR, 0·86; 95% CI, 0·4, 1·82).

3.6 SMFA

Three subjects had 100% TRA/TBA at 10 weeks' post dose 4 (1 control, 2 Pfs230D1 participants). A single subject from main cohort, Pfs230D1, 40µg + saline who had with high, persistent anti-Pfs230D1 titres (1749 EU on day 730) and associated TRA/TBA (100% 10 weeks' post-dose 4) underwent large volume blood draw follow-up visit 12 months post-dose 4.

3.7 Experimental Huts

3.7.1 Summary of Experimental Huts Conducted

In total, 100 EH on 36 unique participants were conducted in 2015 as part of this study protocol. EH conduct was well-accepted by study participants, with 22 study participants undergoing two or more EH procedures. 57 EH (57% of total conducted) yielded captured mosquitoes with an average of 3·3 mosquitoes captured per collection (range 1, 16). In total, 189 mosquitoes were collected over the course of the season of which 143 had a visible bloodmeal (76%).

3.7.2 Results of Assays for Forensic Typing

During the 2015 season, a total of 143 blood-fed mosquitoes were captured, of which 110 were analyzed. Of these 110 mosquitoes, only 40 yielded data using the 16-locus DNA forensic typing kit. 26 of the typed mosquitoes indicated feeding solely on the hut participant (65%), 1 indicated feeding on hut participant plus one other unidentified individual (2·5%) and 13 indicated feeding solely on external individuals (32·5%). Further examination of the 70 mosquitoes that failed to return a forensic typing result showed that only 5 (7%) were positive for human ß-actin, suggesting that the mosquitoes had either fed on a non-human source or the bloodmeal had

degraded and genetic material was no longer detectable. 68/70 (97%) were positive for mosquito 28s DNA suggesting no issues with the DNA extraction process.

3.8 Co-infections

The effect of co-infection on anti-Pfs25, Pfs230D1 and the carrier protein EPA ELISA titres was assessed on days 14 and 42 of the pilot. Urine samples were assayed for *S. haematobium* in the entire pilot cohort. Of the 25 individuals in the pilot, only one infection was noted in the comparator arm. Stool PCR assays were performed on all but 2 participants, and results were grouped into helminth (yes/no) or protozoa (yes/no) by the groupings presented in **Table 1**. Due to the small sample size of the pilot, comparisons of ELISA differences could not be calculated.

In the main study, the impact of co-infection was assessed at days 42 and 182. At the time of publication, 42% of assays were incomplete but spread evenly across treatment groups. Urine analysis revealed 14 *S. haematobium* infections (Pfs230:2/49; Pfs25: 3/50; Pfs25+230: 4/50; Comparator: 5/51). All but one of these infections were mild with one heavily infected case in the Comparator arm. No significant differences in titer were observed between infected and non-infected groups. Stool samples were assayed and mean ELISA titres by protozoa or helminth status were calculated for responses to either vaccine or the carrier. Two weeks post-vaccination 2, co-infection significantly reduced anti-Pfs230D1 titers in the Pfs230D1 arm (p=0·0487), but not 2 weeks post-dose 3. No significant differences were observed in the Pfs25 arm or combination arm.

4 SAMPLE SIZE AND PLANNED ANALYSES

4.1 Safety

The arms of five subjects were sized for safety, as the higher dose was expected to be necessary for an adequate immune response. In these arms, 5 subjects received 15 µg Pfs25M-EPA/Alhydrogel® and/or 16 µg Pfs230D1M-EPA/Alhydrogel®. Vaccination arms of 5 subjects gave a probability of at least 0·80 for detecting 1 or more serious or severe AEs that occur with a probability of 0·275 or more per subject. For each dose level that had a (n=50) main cohort, vaccination of 50 subjects gave a probability of at least 0·90 for detecting 1 or more serious or severe AEs that occur with a probability of 0·045 or more per subject. When combining all treated groups in Mali, 165 subjects who received either Pfs25, Pfs230D1, or Pfs25+Pfs230, we had 95% power to detect 1 or more serious or severe AEs that occur with a probability of 0·018 or more per subject. We compared all AE event proportions between the control arm and treated arm by Fisher's exact test.

4.2 ELISA

There were several questions of interest based on the antibody response information after the 2nd, 3rd and 4th doses of the respective vaccines. We were interested in the change in ELISA values from baseline to after a given number of doses of vaccine, and the change in ELISA values between doses. For this we used Wilcoxon signed rank tests within the 100 subjects receiving a given vaccine. We were also interested in the effect of a given number of vaccinations on ELISA responses compared to placebo. As we did not know whether there would be an interaction effect between the two vaccines, we first ran a linear model with interaction. When examining antigen specific ELISA responses, we expected vaccination with other antigens would have no effect at all. As no interaction was found, we combined all subjects that were given a particular dose level and vaccination type and compared to all subjects that were randomized at the same time and did not receive that vaccination type. This comparison was made by Wilcoxon Mann Whitney test, which accommodated the limit of detection issues that may have existed for ELISA results. We then looked for differences among the treated groups, combined and pairwise.

The preliminary data from our previous study in Mali subjects[8] who had Pfs25 ELISA measurements after receiving two and three doses of 47µg Pfs25, allowed us to estimate the SD of the log-transformed Pfs25-ELISA responses post-vaccination 2 to be 0·91 with a mean of 4·53. Post-3rd vaccination, these same subjects had an estimated SD of the log-transformed Pfs25-ELISA responses of 0·93 and a mean of 5·15. In these same data, all control subjects had undetectable levels of Pfs25 ELISA response post 2nd and 3rd vaccination. This amounted to an observed average 3·3-fold change in geometric mean from the limit of detection post-vaccination 2 and an average 3·98-fold change in geometric mean post-vaccination 3. Assuming that vaccination with Pfs230D1M-EPA/Alhydrogel® had no effect on the level of Pfs25M ELISA responses, we grouped all those that did not receive any Pfs25M vaccination and were randomized at the same time in Mali (100 subjects), and assumed they would be below the limit of detection. We compared to all those subjects that received a Pfs25M vaccination with or without Pfs230D1M in the same Group (100 subjects). Using the background information from our previous study[8], we had greater than 95% power to reject a 2-sided 0·05 level Wilcoxon Mann Whitney test if the geometric mean Pfs25 ELISA level was 1·5-fold higher geometric mean than the level of detection in the vaccinated group post-vaccination 2. Given the similarity in SD estimate post vaccination 3, we had very similar power post-vaccination 3 as was calculated for post-vaccination 2.

Since we did not have information to support the Pfs230D1 power calculations, we used preliminary data from our previous study[8] on the log-transformed EPA-ELISA responses. Subjects with EPA-ELISA measurements after receiving two doses of 47µg Pfs25 allowed us to estimate the SD of the log-transformed EPA-ELISA responses to be 1·1 with a mean of 5·48. In these same data, the control subjects had an estimated mean log EPA-ELISA response of 3·9 and

SD of 0·36 post-2nd placebo injection. We used EPA responses for these calculations, as it is possible that control subjects had detectable Pfs230D1 responses at some point during the trial. Therefore, if we based the Pfs230D1 ELISA response power calculations on the Pfs25 responses above, this would be anti-conservative as it assumes zero variation in the control group responses. We based all further ELISA power calculations on EPA-response-based simulations for this reason.

In the very unlikely case that there was an interaction effect of 2-fold or greater in the geometric mean, we would have had 80% power to detect that after the 2nd vaccination, given the simulations assumptions and using a linear interaction model. Assuming no effect of Pfs25 vaccination on Pfs230D1 titres, and simulating data using these SD estimates and the mean from the control group for EPA responses in our previous study[8], for 100 subjects per arm, we found 85% power to reject at the 2-sided 0·05 level via Wilcoxon Mann Whitney test if the geometric mean ELISA level was 1·45-fold higher in vaccinated group.

We were also interested in testing for differences in antibody response, possibly for EPA, between the treated arms. We used a Kruskal-Wallis test for differences over the three treated groups; upon rejection by this test, we moved on to the pairwise Wilcoxon Mann Whitney tests between each group. Under no interaction and equal and positive treatment effect in each group, the combined group median would have still been higher than either of the single treatment groups, and would simply be the addition of the two treatment effects. Given our simulation assumptions, based on the EPA responses post-vaccination 2, we had approximately 80% power to detect a difference in median over the treated groups if each treatment had 1·83-fold increased geometric mean response from the placebo group and there was no interaction. Clearly power would have increased if each vaccination type had a different treatment effect on the ELISA response of interest, or if there was an interaction effect. For the pairwise comparisons in this case, we would have had 80% power to detect a difference using a 2-side 0·05 Wilcoxon Mann Whitney test, 50 subjects to 50 subjects, of 1·85-fold or more in geometric mean or more between any of the treatment groups.

To consider power after the third vaccination, we again used data from our previous study[8] on 45 subjects who had measurements after receiving three doses of 47μg Pfs25. In this group, the SD of the log-transformed EPA-ELISA responses was estimated to be 0·65 with a mean of 6·15. In these same data, the control subjects had an estimated mean log EPA-ELISA response of 3·88 and SD of 0·36.

If there was an interaction effect of 1·63-fold or greater in geometric mean, we would have had 80% power to detect that after the 3rd vaccination, given the simulations assumptions and using a

linear interaction model. Again, assuming no effect of vaccination by Pfs25 on Pfs230D1 titres and simulating data using the post-3rd vaccination EPA-response based estimate, for 100 subjects per arm, we expected to have greater than 85% power to detect a 1·27 fold increase in geometric mean ELISA response for a two-sided 0·05 Wilcoxon Mann Whitney test.

To compare the treated groups, we found a 2-sided 0·05 Kruskal-Wallis test should have had approximately 80% power to reject the null of no difference in medians after the third vaccination if each treatment had 1·43-fold increase in geometric mean from the placebo group and there was no interaction. For the pairwise comparisons in this case, we had 80% power to detect a pairwise difference using a 2-side 0·05 Wilcoxon Mann Whitney test, 50 subjects to 50 subjects, of 1·45-fold or more in geometric mean between any of the treatment groups.

To consider power for the group over time comparisons, we again used the same EPA titre based data as a basis of our simulations. We found that we should have had 80% power to reject the null of no difference of medians using a 2-sided 0·05 Wilcoxon signed rank test with a 1·45-fold difference in geometric mean from post-vaccination 2 to post-vaccination 3 in the 100 subjects that received a given vaccine. For the post-2nd vaccination comparison to baseline, using data from our previous study[8] that estimates a baseline SD of 0, we found greater than 80% power to detect a 1·4-fold increase in geometric mean from baseline. To compare post-vaccination 3 to baseline, we found greater than 80% to detect a 1·21-fold increase in geometric mean from baseline.

As we did not have data post 4th vaccination with which to inform power calculations, we did not attempt to extrapolate. However, if the post 4th vaccination data had a SD less than that assumed for the 3rd vaccination, which was the trend between the 2nd and 3rd vaccinations, power would have increased.

Antibody decay profiles for Pfs230D1 and Pfs25 were modeled with a hierarchical Bayesian model. Durability of antibody titres was modeled using a hierarchical Bayesian model fit separately to Pfs230D1 and Pfs25 arms. Geometric mean peak antibody responses at the 2 week timepoint following each dose were modeled using a multilevel Bayesian model with fixed effects for treatment arm, number of doses (treated as a categorical variable), and their interaction, along with an offset for the batch LoD of the assay. Intra-subject correlation was incorporated via nested random intercepts for each participant and number of doses within each participant's time series, and batch effects were incorporated via random intercepts for each plate. Antibody waning was modeled using Weibull decay profiles, which accommodate time-inhomogeneity in the rate of decay. The (log) shape and (logit) scale parameters of the decay profile were regressed on treatment arm and constrained to reflect that antibodies wane over time

and that the rate of decay slows as a function of time since peak titre. Titres that were below the batch LoD were censored at the batch LoD. Conditional on the mean model governing antibody response and decay, errors modeled as normally distributed with standard deviation depending on the number of doses received, (log) time since the previous peak response, and their interaction. The models were fit using weakly informative priors using the brms package in R.[9] As a diagnostic, we compared the posterior predictive distributions of Pfs230D1 and Pfs25 antibody titres to the raw data with measurements below the LoD crudely imputed at half the LoD. Convergence and mixing diagnostics for MCMC included visual inspection of MCMC traceplots of all model parameters as well as calculation of effective sample sizes and potential scale reduction factors.

The number of doses needed to elicit an immune response was defined in this supplementary analysis for each participant by counting the number of doses until antibody titre exceeded a pre-defined threshold, based on observed titres in the TWINRIX comparator arm. For Pfs25, the threshold for declaring an immune response was set equal to the maximum observed titre in the comparator arm, after adjusting for batch effects in the limit of detection (LoD). Two percent of participants in the Twinrix arm had a nominal titre greater than 50 ELISA units at baseline, reflecting an immune response to natural falciparum infection. Hence, the threshold for declaring a Pfs230D1 immune response was set to the 98th percentile of baseline Pfs230D1 titre measurements in the Twinrix arm, after adjusting for batch effects in the LoD. These cutoffs represent less stringent thresholds than those used to declare seroconversion in the presentation of the raw data. Assay batch effects were adjusted for by subtracting the plate LoD from the measured natural log ELISA titres. The cutoffs for Pfs230D1 and Pfs25, respectively, corresponded to increases of 0·21 and 0·041 $\log_{10}$ ELISA units relative to the batch limit of detection (LoD).

To additionally assess immunogenicity, two Bayesian proportional odds models were fit for the number of doses required to elicit an immune response, as defined in the previous paragraph. The models were fit separately for Pfs230D1 and Pfs25 arms, with one model comparing Pfs25 against the combination arm, and the other comparing Pfs230D1 against combination vaccination. The treatment effect in each model is a common odds ratio (COR). For any number of doses, the COR contrasts the odds of needing more than that number of doses to elicit a response versus that number or fewer in the Pfs230D1 or Pfs25 arms compared with the combination vaccination arm. By proportional odds, the COR is constrained to equality for all possible choices of reference dose number. In our models, a COR less than 1 indicates that administering the vaccines in combination requires fewer doses to achieve an immune response. We assigned a standard uniform prior on the proportion of variance explained by treatment arm, and a uniform Dirichlet distribution on the number of doses needed to elicit a response. We fit

the models using the rstanarm package in R[7] and refer to the rstanarm documentation for details about parameterization and prior specification.

4.3 Functional Assays

Using the data from the primary DSF evaluation period of our previous study[8] we were able to perform power calculations for this study. Given that 6/38 subjects in the control arm of our previous study[8] had at least one positive DSF that is ~16% infectivity. Using this same zero-inflation rate and comparing 100 controls to 100 subjects per vaccine type we had ~80% power to detect 80% vaccine effect through the (0,1) infective/non-infective way of quantifying DSF with 12 DSF per person.

We based power on a beta-binomial model fit to the data in the comparator group alone during the 6-week primary observation period in our previous study,[8] post-vaccination #4. The power simulations fit a method of moments beta distribution to the proportion of infected mosquitoes averaged within subject. We used Beta distribution governed by the estimates from our data, to generate a probability for each of the 200 subjects that 1 of their N mosquitoes is infected, during the K weeks. We then zero-inflated those subject level probabilities, holding it constant along with probabilities per-subject over the full follow-up. This zero-inflation rate was estimated from the previous study[8] data. We then tested for differences between arms for the output of infected mosquitoes/dissected over all time points by logistic GEE, and again by (0,1) ever having a positive DSF by Fisher's exact test. We used this data simulated under this model to investigate how the DSF procedure can be changed to increase power, increasing the number of mosquitoes per jar and increasing the number of feeds during the follow-up period.

Since it was difficult using this model to pinpoint the exact power and effect size of interest, we instead investigated the type of increase we would see given the changes to the procedure. The model suggested that increasing the number of mosquitoes would have very little effect on power because the beta distribution is multimodal, with a large number of subjects very near 0 probability and a small number much higher. With a ~80% VE for the (0,1) infectivity and with ~80% VE using a logistic GEE model, we found that increasing from 60 mosquitoes per DSF to 120 only produced a 2% increase in power. The minor increase in power when we doubled the count is due to the estimated Beta distribution. When we doubled the number of mosquitoes in a feed, we get those subjects with high probabilities having higher infected counts, but the other subjects remained almost the same. However, using this model to simulate an additional 6 feeds per person, we saw that under the same 80% VE setting that we would get ~15% gain in power. For both scenarios, power increases seen decreased as overall power increases, as there is less room for improvement.

If instead we generated a random beta for each feeding week for 100 subjects, we would have already had moderately good power (~70% for 80% VE). However, we again found that we

gained more power by increasing the number of feeds rather than the number of mosquitoes, with the gain being ~17% for 12 feeds per person, yielding >80% power, while we saw again that increasing to 120 mosquitoes per feed only yielded ~4% more power.

For this reason, we planned to increase the number of DSF run per-person to as many as 12 over the 6-week primary analysis period post the last vaccination. Based on the full group under Pfs25 or Pfs230D1 versus the full group that did not receive Pfs25 or Pfs230d1, respectively ~100 versus 100, we should have had >80% power to detect an 80% vaccine effect on DSF, assuming a zero-inflation rate of ~84% and performing ~12 feeds per subject over 6 weeks.

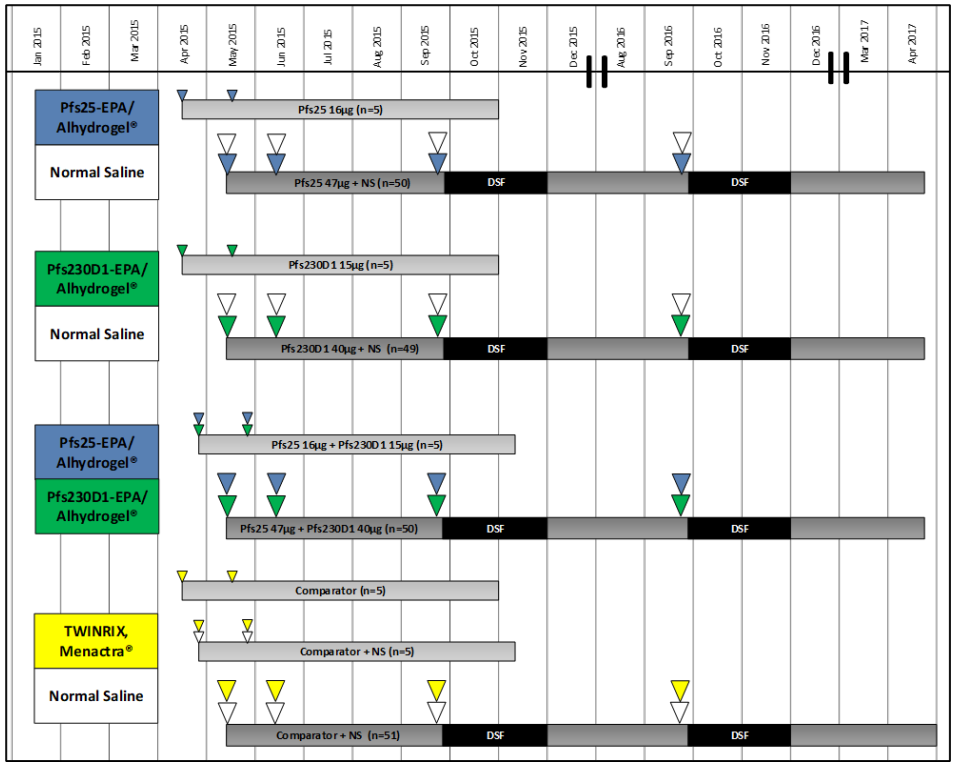
We also reduced the number of mosquitoes per cup for the DSFs post-vaccination 4. The justification for moving to fewer mosquitoes was given by the power simulations which changed less than 2% when going from 60 to 30 mosquitoes. This is because we used a model that accounted for the number of mosquitoes dissected, so we were most interested in the proportion not the absolute number of infected mosquitoes. As well, to check these simulation results empirically using the data from our previous study,[8] we randomly selected 15 mosquitoes from each cup for a given feed without replacement. We conducted this simulation 500 times and calculated the beta parameters and the zero-inflation rate each time. On average, using 15 rather than 30 mosquitoes per cup did not significantly change the parameters of the beta distribution on average; in fact it made the beta slightly better. The median of the zero-inflation rate over these resampled sets was also not significantly different from the use of 30 mosquitoes per cup.

Due to the number of tests performed, p-value adjustment was considered to control the type 1 error rate.

5 TABLES AND FIGURES

5.1 Figure S1. Phase 1 study of Pfs25-EPA/Alhydrogel® and Pfs230D1-EPA/Alhydrogel® in Malian healthy adults: study schema

Figure is representative of actual vaccinations and DSF time periods. Upside down triangles indicate timing of vaccinations with blue arrows = Pfs25-EPA/Alhydrogel®, green arrows = Pfs230D1-EPA/Alhydrogel®, yellow arrows = comparator vaccine (TWINRIX for dose 1, 2, 3 and Menactra® for dose 4), and white arrows = normal saline. Size of arrows are indicative of dosing of Pfs25 (small = 16µg, large = 47µg) and Pfs230D1 (small = 15µg, large = 40µg). DSF = direct skin feeds. EPA = ExoProtein A.



5.2 Table S1. Solicited local and systemic reactogenicity and safety laboratories.

After each vaccination, subjects were monitored for at least 30 minutes for local and systemic reactogenicity. Subjects were evaluated on site for safety on days 1, 3, 7, 14, and 28 following vaccination and monthly during the long-term safety follow-up. Medically qualified study personnel were available at all times for unscheduled visits. Solicited local and systemic reactogenicity events were documented for 7 days (local) and 14 days (systemic) after vaccination. Protocol-specified laboratory assessments, including complete blood count with differential, creatinine level, alanine aminotransferase (ALT) levels were completed prior to each vaccination and on days 3 and 14 after each vaccination. Laboratory abnormalities were collected within 28 days of vaccination as noted but were not considered solicited.

Systemic adverse events	Laboratory adverse events	Local reactogenicity
Fever (temperature ≥38·0 °C)	Hemoglobin (low hemoglobin, decreased hemoglobin)	Injection pain/tenderness
Headache (a pain located in the head, over the eyes, at the temples, or at the base of the skull and lasting more than 30 minutes)	WBC (leukopenia, leukocytosis)	Injection erythema/redness
Nausea (discomfort in the stomach with an urge to vomit)	ANC/AGC (neutropenia, granulocytopenia)	Injection swelling
Malaise (generalized feeling of being unwell)/Fatigue	Platelet count (thrombocytopenia)	Injection induration
Myalgia (pain in the muscles, in one or more muscle groups)	ALT (increased ALT)	Injection pruritus
Arthralgia (pain in a joint, in one or more joints)	Creatinine (increased creatinine)	
Urticaria (hives; a raised, red, itchy skin rash containing wheals)		

5.3 Table S2. Local normal laboratory values with healthy Malian adults.

The laboratory values provided in the table are based on Bancoumana, Malian adult normal (age 18-45 years old).

Laboratory	Reference Range
Hemoglobin (female) - gm/dL	9·1 – 13·8
Hemoglobin (male) - gm/dL	10·8 – 15·8
White blood cell - 10^3/µL	3·6 – 9·0
Absolute neutrophil or granulocyte count - 10^3/µL	1·3 – 4·4
Platelet count (female)- 10^3/µL	144 – 413
Platelet count (male)- 10^3/µL	114 – 335
Creatinine (female) - µmol/L	< 72
Creatinine (male) -µmol/L	48 – 98
Alanine aminotransferase – U/L	< 41

5.4 Table S3. Toxicity grading scale for laboratory parameters.

Grading of AEs were based on FDA toxicity grading and adjusted based on local normal laboratory values. gm/dL = grams/deciliter; µL = microliters; µmol/L = micromoles/liter; U/L = units/liter; ULN = upper limit of normal. N/A = not applicable.

Hematology and Biochemistry values	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male) - gm/dL	9·5 – 10·5	8·0 – 9·4	6·5 – 7·9	< 6·5 and / or requiring transfusion
Hemoglobin (Female) gm/dL	8·0 – 9·0	7·0 – 7·9	6·0 – 6·9	< 6 and /or requiring transfusion
WBC Increase – 10^3/µL	11·5 – 15·0	15·1 – 20·0	20·1 – 25·0	> 25·0
WBC Decrease - 10^3/µL	2·5 – 3·3	1·5 – 2·4	1·0 – 1·4	< 1·0 with fever
Neutrophil/Granulocyte Decrease - 10^3/µL	0·8 – 1·0	0·5 – 0·7	< 0·5	< 0·5 with fever
Platelets Decreased – 10^3/µL	100 – 115	70 – 99	25 – 69	< 25
Creatinine (Male) µmol/L	124·00 – 150·99	151·00 – 176·99	177·00 – 221·00	> 221·00 and requires dialysis
Creatinine (Female) µmol/L	107·00 – 132·99	133·00 – 159·99	160·00 – 215·99	> 216·00 and requires dialysis
Liver Function Tests –ALT U/L	75·0 – 150·9	151·0 – 300·9	301·0 – 600·0	> 600·0

5.5 Table S4. Immunogenicity and functional activity timepoints.

Tables representing sampling timeline (by day, week, month) in relation to day of vaccination (blue) and DSF evaluation (orange). Blue = dates of vaccination; orange = time period of DSF, green = assay ran in available samples; yellow = SMFA result only available on a subset of individuals (selected individuals who had >90% TRA activity on study day 182; note some samples were excluded from being included). Y= yes, Y* = yes to subset, N= no. Vax = vaccination. Grey italized text = not all subjects had sample collected on those study days. A) Pilot safety cohort, Pfs25, 16 µg; Pfs230D1, 15 µg; Pfs25 16 µg + Pfs230D1, 15 µg; and TWINRIX + NS. Vaccinations were administered on a 0, 1 month schedule from April to May 2015. B) Main cohort, Pfs25, 47 µg + NS; Pfs230D1, 40 µg + NS; Pfs25 47 µg + Pfs230D1, 40 µg; and TWINRIX/Menactra + NS. Vaccinations were administered on a 0, 1, 4·5, 16·5 month schedule from May to October 2015 (for dose 1, 2, 3) and September to October 2016 (for dose 4).

A) Pilot Safety Cohort

				Pfs25				Pfs230				Pfs25 + Pfs230				Twinrix +/- NS			
				ELISA				ELISA				ELISA				ELISA			
				Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA
Study Days (ELISA + SMFA)	Days Post Vaccination	Approximate Months Post Vaccination (by protocol)	Approximate Weeks Post Vaccination																
0	Vax 1	0	0	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
14	14 days post Vax 1	0.5	2	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
28	Vax 2	0	0	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
42	14 days post Vax 2	0.5	2	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
84	56 days post Vax 2	2	8	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
140	112 days post Vax 2	4	16	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
196	168 days post Vax 2	6	24	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N	Y	Y	N	N

B) **Main Cohort**

						47 ug Pfs25 + NS				40 ug Pfs230 + NS				47 ug Pfs25 + 40 ug Pfs230				Twinrix, Menactra + NS			
						ELISA				ELISA				ELISA				ELISA			
						Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA	Pfs25	Pfs230	EPA	SMFA
Study Days (ELISA + SMFA)	Days Post Vaccination	Approximate Months Post Vaccination (by protocol)	Approximate Weeks Post Vaccination	Scheduled Collection on All?	During DSF Evaluation																
0	Vax 1	0	0	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
14	14 days post Vax 1	0.5	2	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
28	Vax 2	0	0	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
42	14 days post Vax 2	0.5	2	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
84	56 days post Vax 2	2	8	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
140	112 days post Vax 2	4	16	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
168	Vax 3		0	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
175	7 days post Vax 3	0.25	1	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
182	14 days post Vax 3	0.5	2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
196	28 days post Vax 3	1	4	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
210	42 days post Vax 3	1.5	6	Y	Y	Y	Y	Y	Y*	Y	Y	Y	Y*	Y	Y	Y	Y*	Y	Y	Y	Y*
217	*49 days post Vax 3*	*1.75*	*7*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
240	72 days post Vax 3	~2	~10	Y	N	Y	N	N	N	N	Y	N	N	Y	Y	N	N	N	N	N	N
270	*102 days post Vax 3*	*~3*	*~15*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
300	132 days post Vax 3	~4	~19	Y	N	Y	N	N	N	N	Y	N	N	Y	Y	N	N	Y	Y	N	N
330	*162 days post Vax 3*	*~5*	*~23*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
360	192 days post Vax 3	~6	~27	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
390	*222 days post Vax 3*	*~7*	*~32*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
420	252 days post Vax 3	~8	~36	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
450	*282 days post Vax 3*	*~9*	*~40*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
480	312 days post Vax 3	~10	~45	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
510	*342 days post Vax 3*	*~11*	*~49*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
540	Vax 4	0	0	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N	Y	Y	Y	N
547	7 days post Vax 4	0.25	1	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
554	14 days post Vax 4	0.5	2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
568	28 days post Vax 4	1	4	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
582	42 days post Vax 4	1.5	6	Y	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
589	*49 days post Vax 4*	*1.75*	*7*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
610	70 days post Vax 4	~2	10	Y	N	Y	N	N	N	N	Y	N	Y	Y	Y	N	N	Y	Y	N	Y
640	*100 days post Vax 4*	*~3*	*~14*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
670	130 days post Vax 4	~4	~19	Y	N	Y	N	N	N	N	Y	N	N	Y	Y	N	N	Y	Y	N	N
700	*160 days post Vax 4*	*~5*	*~23*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
730	190 days post Vax 4	~6	~27	Y	N	Y	N	N	N	N	Y	N	N	Y	Y	N	N	N	N	N	N
910	*370 days post Vax 4*	*~12*	*~53*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*

5.6 Table S5. Pilot safety cohort local and systemic reactogenicity

Local reactogenicity was assessed until 7 days post vaccination; solicited reactogenicity was assessed until 14 days post vaccination. Local injection site reactogenicity included: pain/tenderness, erythema/redness, swelling, induration, and pruritus. Systemic solicited reactogenicity included: fever, headache, nausea, malaise, myalgia, arthralgia, and urticaria. For subjects receiving two vaccinations (co-administration arms, Pfs25, 16 µg + Pfs230D1, 15 µg and TWINRIX + NS) if local reactogenicity reported the attributed vaccine responsible for the local reaction is specified below. Vaccinations were administered on a 0, 1 month schedule from April to May 2015. All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. AE = adverse events; µg = micrograms. NS = normal saline. All local reactogenicity and all solicited reactogenicity reported were Grade 1 (mild).

	Pfs25, 16 µg		**Pfs230, 15 µg**		**Pfs25, 16 µg + Pfs230, 15 µg**				**TWINRIX +/- Normal Saline**			
					Vax 1 (N=5)		**Vax 2 (N=5)**		**Vax 1 (N=10)**		**Vax 2 (N=10)**	
	Vax 1 (N=5)	**Vax 2 (N=5)**	**Vax 1 (N=5)**	**Vax 2 (N=4)**	***Vaccine local reactogenicity attributed to***				***Vaccine local reactogenicity attributed to***			
					Pfs25, 16 µg	**Pfs230, 15 µg**	**Pfs25, 16 µg**	**Pfs230, 15 µg**	**TWINRIX**	**NS**	**TWINRIX**	**NS**
Local Reactogenicity	2 (40%) 2	2 (40%) 2	0 (0%) 0	0 (0%) 0	3 (60%) 5		2 (40%) 3		1 (10%) 1		3 (30%) 3	
Injection site pain/tenderness	2 (40%) 2	2 (40%) 2	0 (0%) 0	0 (0%) 0	1 (20%) 1	2 (40%) 2	1 (20%) 1	2 (40%) 2	0 (0%) 0	1 (10%) 1	3 (30%) 3	0 (0%) 0
Injection site erythema/redness	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection swelling/edema	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection induration	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection pruritus	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (20%) 1	1 (20%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

	Pfs25, 16 µg		Pfs230, 15 µg		Pfs25, 16 µg + Pfs230, 15 µg		TWINRIX +/- NS	
	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=5)	Vax 2 (N=4)	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=10)	Vax 2 (N=10)
Solicited Reactogenicity	0 (0%) 0	0 (0%) 0	1 (20%) 1	0 (0%) 0	1 (20%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Fever	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Headache	0 (0%) 0	0 (0%) 0	1 (20%) 1	0 (0%) 0	1 (20%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Nausea	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Malaise	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Myalgia	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Arthralgia	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Urticaria	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

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5.7 Table S6. Pilot safety cohort laboratory abnormalities.

Laboratory AEs were assessed until 14 days post vaccination + visit window timeframe (+3 days). Scheduled labs (complete blood cell count with differential, alanine transaminase (ALT), creatinine) were completed on day of vaccination and then 3 and 14 days post vaccination. Laboratory adverse events were collected for the following: hemoglobin decreased, thrombocytopenia, leukocytosis, leukopenia, neutropenia, ALT increase, blood creatinine increased and for any medically important laboratory abnormality (at the discretion of the investigator). Vaccinations were administered on a 0, 1 month schedule from April to May 2015. Follow-up concluded by November 2015. All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. Vax = Vaccination. AE = adverse events; µg = micrograms. [A]Both Grade 4 laboratory AEs occurred in the same subject and is summarized in **Section 3.1.1** of the supplemental appendix.

	Pfs25, 16 µg		Pfs230, 15 µg		Pfs25, 16 µg + Pfs230, 15 µg		TWINRIX +/- NS	
	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=5)	Vax 2 (N=4)	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=10)	Vax 2 (N=10)
Laboratory AEs	0 (0%) 0	1 (20%) 1	1 (20%) 2	2 (50%) 2	1 (20%) 1	1 (20%) 1	1 (10%) 1	1 (10%) 1
Grade 1	0 (0%) 0	1 (20%) 1	0 (0%) 0	2 (50%) 2	1 (20%) 1	1 (20%) 1	1 (10%) 1	0 (0%) 0
Grade 2	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (10%) 1
Grade 3	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Grade 4	0 (0%) 0	0 (0%) 0	1 (20%) 2[A]	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Individual Laboratory AEs								
Anemia/Hemoglobin Decreased	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Leukopenia	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (25%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Leukocytosis	0 (0%) 0	0 (0%) 0	1 (20%) 1[A]	1 (25%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Neutropenia	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (20%) 1	1 (20%) 1	0 (0%) 0	0 (0%) 0
Thrombocytopenia	0 (0%) 0	1 (20%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (10%) 1	1 (10%) 1
Blood Creatinine Increased	0 (0%) 0	0 (0%) 0	1 (20%) 1[A]	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
ALT Increased	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

5.8 Table S7. Safety summary of pilot safety cohort.

Reporting periods for adverse events (AEs) were protocol specific. Unsolicited AEs, serious AEs (SAEs), unanticipated problems (UPs), and (new onset chronic illness (NOCIs) were recorded through the end of the study (study day 196, ~6 months post vaccination #2). The following reporting periods were defined as follows: during entire study period (for Vax 1 = ~1 month, Vax 2 = ~5 months); local reactogenicity was assessed until 7 days post vaccination; solicited reactogenicity was assessed until 14 days post vaccination; laboratory AEs were assessed until 14 days post vaccination + visit window timeframe (+3 days). Local injection site reactogenicity included: pain/tenderness, erythema/redness, swelling, induration, and pruritus. Systemic solicited reactogenicity included: fever, headache, nausea, malaise, myalgia, arthralgia, and urticaria. Scheduled labs (complete blood cell count with differential, alanine transaminase, creatinine) were completed 3 and 14 days post vaccination. For subjects receiving two vaccinations (co-administration arms, Pfs25, 16 µg + Pfs230D1, 15 µg and TWINRIX + NS) if local reactogenicity reported and attributed to both upper arms, two individual AEs are reported in one subject. Symptomatic malaria was reported as an AE (defined as *Plasmodium* asexual parasitaemia accompanied by an axillary temperature of at least 37·5 °C and/or clinical signs and symptoms compatible with malaria) and collected throughout the study duration. Vaccinations were administered on a 0, 1 month schedule from April to May 2015. Follow-up concluded by November 2015. All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. Vax = Vaccination. AE = adverse events; SAE = serious adverse events. µg = micrograms. [A]All three reported Grade 3 (N=1) and Grade 4 (N=2) AEs occurred in the same subject and is summarized in **Section 3.1.1** of the supplemental appendix.

	Pfs25, 16 µg		Pfs230, 15 µg		Pfs25, 16 µg + Pfs230, 15 µg		TWINRIX +/- NS	
	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=5)	Vax 2 (N=4)	Vax 1 (N=5)	Vax 2 (N=5)	Vax 1 (N=10)	Vax 2 (N=10)
Reported during entire study period								
Total AE	4 (80%) 5	5 (100%) 14	4 (80%) 7	4 (100%) 10	4 (80%) 9	5 (100%) 16	4 (40%) 10	9 (90%) 20
Grade 1	2 (40%) 2	4 (80%) 5	2 (40%) 2	4 (100%) 4	4 (80%) 7	5 (100%) 10	3 (30%) 5	5 (50%) 6
Grade 2	2 (40%) 3	5 (100%) 9	2 (40%) 2	2 (50%) 6	2 (40%) 2	3 (60%) 5	2 (20%) 3	7 (70%) 13
Grade 3	0 (0%) 0	0 (0%) 0	1 (20%) 1	0 (0%) 0	0 (0%) 0	1 (20%) 1	0 (0%) 0	1 (10%) 1
Grade 4	0 (0%) 0	0 (0%) 0	1 (20%) 2[A]	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Grade 5	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Related AE	2 (40%) 2	2 (40%)2	1 (20%) 1	1 (25%) 1	4 (80%) 5	3 (60%) 4	1 (10%) 1	3 (30%) 3
SAE	0 (0%) 0	0 (0%)0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Malaria AE	0 (0%) 0	1 (20%) 1	0 (0%) 0	2 (50%) 2	0 (0%) 0	4 (80%) 6	0 (0%) 0	4 (40%) 5
Reported within 7 days of vaccination								
Local Reactogenicity	2 (40%) 2	2 (40%) 2	0 (0%) 0	0 (0%) 0	3 (60%) 5	2 (40%) 3	1 (10%) 1	3 (30%) 3
Reported within 14 days of vaccination								
Solicited Reactogenicity	0 (0%) 0	0 (0%) 0	1 (20%) 1	0 (0%) 0	1 (20%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Laboratory AE	0 (0%) 0	1 (20%) 1	1 (20%) 2	2 (50%) 2	1 (20%) 1	1 (20%) 1	1 (10%) 1	1 (10%) 1

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5.9 Table S8. Main cohort local reactogenicity.

Local reactogenicity was assessed until 7 days post vaccination. Local injection site reactogenicity included: pain/tenderness, erythema/redness, swelling, induration, and pruritus. Given all subjects received two vaccinations (co-administration), if local reactogenicity reported the attributed vaccine responsible for the local reaction is specified below. Vaccinations were administered on a 0, 1, 4·5, 16·5 month schedule from May to October 2015 (for dose 1, 2, 3) and September to October 2016 (for dose 4). All local reactogenicity was either Grade 1 or Grade 2. All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. AE = adverse events. NS= normal saline. µg = micrograms. Significant differences from the control are noted with an *.

	Pfs25, 47 µg + NS								**Pfs230, 40 µg + NS**							
	Vax 1 (N=50)		**Vax 2 (N=48)**		**Vax 3 (N=44)**		**Vax 4 (N=42)**		**Vax 1 (N=49)**		**Vax 2 (N=45)**		**Vax 3 (N=43)**		**Vax 4 (N=40)**	
	Vaccine local reactogenicity attributed to								***Vaccine local reactogenicity attributed to***							
	Pfs25, 47 µg	**NS**	**Pfs25, 47 µg**	**NS**	**Pfs25, 47 µg**	**NS**	**Pfs25, 47 µg**	**NS**	**Pfs230, 40 µg**	**NS**	**Pfs230, 40 µg**	**NS**	**Pfs230, 40 µg**	**NS**	**Pfs230, 40 µg**	**NS**
Local Reactogenicity	26* (52%) 32		31* (64·6%) 42		16* (36·4%) 23		20* (47·6%) 23		21* (42·9%) 24		22 (48·9%) 22		17* (39·5%) 21		16 (40%) 16	
Grade 1	26* (52%) 31		29* (60·4%) 33		16* (36·4%) 20		10 (23·8%) 12		20* (40·8%) 23		21 (46·7%) 21		17* (39·5%) 21		15 (37·5%) 15	
Grade 2	1 (2%) 1		9 (18·8%) 9		2 (4·5%) 3		10 (23·8) 11		1 (2%) 1		1 (2·2%) 1		0 (0%) 0		1 (2·5%) 1	
Individual Local Reactogenicity																
Injection site pain/tenderness	21* (42%) 21	9 (18%) 9	30* (62·5%) 30	2 (4·2%) 2	16* (36·4%) 16	3 (6·8%) 3	17 (40·5%) 17	5 (11·9%) 6	19* (38·8%) 19	4 (8·2%) 4	21* (46·7%) 21	0 (0%) 0	16* (37·2%) 16	5 (11·6%) 5	15 (37·5%) 15	1 (2·5%) 1
Injection site erythema/redness	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection swelling/edema	0 (0%) 0	0 (0%) 0	3 (6·3%) 3	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection induration	1 (2%) 1	0 (0%) 0	6 (12·5%) 6	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	1 (2·2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection pruritus	0 (0%) 0	0 (0%) 0	1 (2·1%) 1	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

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	Pfs25, 47 µg + Pfs230, 40 µg								TWINRIX/Menactra + NS							
	Vax 1 (N=50)		Vax 2 (N=50)		Vax 3 (N=46)		Vax 4 (N=39)		Vax 1 (N=51)		Vax 2 (N=47)		Vax 3 (N=44)		Vax 4 (N=40)	
	Vaccine local reactogenicity attributed to								*Vaccine local reactogenicity attributed to*							
	Pfs25, 47 µg	Pfs230, 40 µg	Pfs25, 47 µg	Pfs230, 40 µg	Pfs25, 47 µg	Pfs230, 40 µg	Pfs25, 47 µg	Pfs230, 40 µg	TWINRIX	NS	TWINRIX	NS	TWINRIX	NS	Menactra	NS
Local Reactogenicity	26* (52%) 48		23 (46%) 43		22* (47·8%) 40		17 (43·6%) 28		6 (11·8%) 9		14 (29·8%) 16		4 (9·1%) 4		10 (25%) 15	
Grade 1	25* (50%) 47		23 (46%) 43		22* (47·8%) 37		10 (25·6%) 16		6 (11·8%) 9		14 (29·8%) 16		4 (9·1%) 4		5 (12·5%) 7	
Grade 2	1 (2%) 1		0 (0%) 0		3 (6·5%) 3		8 (20·5%) 12		0 (0%) 0		0 (0%) 0		0 (0%) 0		6 (15%) 8	
Individual Local Reactogenicity																
Injection site pain/tenderness	22* (44%) 22	23* (46%) 23	16 (32%) 16	18 (36%) 18	17 (37%) 17	21* (45·7%) 21	14 (35·9%) 14	14 (35·9%) 14	5 (9·8%) 5	4 (7·8%) 4	11 (23·4%) 11	3 (6·4%) 3	2 (4·5%) 2	2 (4·5%) 2	10 (25%) 10	3 (7·5%) 3
Injection site erythema/redness	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Injection swelling/edema	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	1 (2·2%) 1	1 (2·2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·5%) 1	0 (0%) 0
Injection induration	1 (2%) 1	2 (4%) 2	3 (6%) 3	5 (10%) 5	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·1%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·5%) 1	0 (0%) 0
Injection pruritus	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·1%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

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1369

5.10 Table S9. Main cohort solicited reactogenicity.

Systemic solicited reactogenicity included: fever, headache, nausea, malaise, myalgia, arthralgia, and urticaria; solicited reactogenicity was assessed until 14 days post vaccination. Vaccinations were administered on a 0, 1, 4·5, 16·5 month schedule from May to October 2015 (for dose 1, 2, 3) and September to October 2016 (for dose 4). All solicited reactogenicity were either Grade 1 or Grade 2; no reported Grade 3, 4, 5. All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. AE = adverse events. Significant differences from the control are noted with an *.

	Pfs25, 47 µg + NS				Pfs230, 40 µg + NS				Pfs25, 47 µg + Pfs230D1, 40 µg				TWINRIX/Menactra + NS			
	Vax 1 (N=50)	**Vax 2 (N=48)**	**Vax 3 (N=44)**	**Vax 4 (N=42)**	**Vax 1 (N=49)**	**Vax 2 (N=45)**	**Vax 3 (N=43)**	**Vax 4 (N=40)**	**Vax 1 (N=50)**	**Vax 2 (N=50)**	**Vax 3 (N=46)**	**Vax 4 (N=39)**	**Vax 1 (N=51)**	**Vax 2 (N=47)**	**Vax 3 (N=44)**	**Vax 4 (N=40)**
Solicited Reactogenicity	3 (6%) 3	3 (6·3%) 3	4 (9·1%) 4	3 (7·1%) 4	5 (10·2%) 5	3 (6·7%) 3	1 (2·3%) 1	5 (12·5%) 5	2 (4%) 2	3 (6%) 3	2 (4·3%) 4	8* (20·5%) 8	5 (9·8%) 5	4 (8·5%) 5	2 (4·5%) 3	1 (2·5%) 1
Grade 1	2 (4%) 2	1 (2·1%) 1	2 (4·5%) 2	1 (2·4%) 1	1 (2%) 1	1 (2·2%) 1	1 (2·3%) 1	3 (7·5%) 3	0 (0%) 0	2 (4%) 2	2 (4·3%) 4	4 (10·3%) 4	2 (3·9%) 2	4 (8·5%) 5	1 (2·3%) 1	0 (0%) 0
Grade 2	1 (2%) 1	2 (4·2%) 2	2 (4·5%) 2	2 (4·8%) 3	4 (8·2%) 4	2 (4·4%) 2	0 (0%) 0	2 (5%) 2	2 (4%) 2	1 (2%) 1	0 (0%) 0	4 (10·3%) 4	3 (5·9%) 3	0 (0%) 0	2 (4·5%) 2	1 (2·5%) 1
Individual Solicited Reactogenicity																
Fever	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·4%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·5%) 1	0 (0%) 0	1 (2%) 1	1 (2·2%) 1	1 (2·6%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Headache	2 (4%) 2	2 (4·2%) 2	4 (9·1%) 4	2 (4·8%) 2	2 (4·1%) 2	2 (4·4%) 2	0 (0%) 0	2 (5%) 2	2 (4%) 2	2 (4%) 2	2 (4·3%) 2	5 (12·8%) 5	2 (3·9%) 2	4 (8·5%) 4	2 (4·5%) 2	1 (2·5%) 1
Nausea	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·5%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Malaise	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	1 (2·2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·6%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Myalgia	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Arthralgia	0 (0%) 0	1 (2·1%) 1	0 (0%) 0	1 (2·4%) 1	1 (2%) 1	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·2%) 1	1 (2·6%) 1	2 (3·9%) 2	1 (2·1%) 1	0 (0%) 0	0 (0%) 0
Urticaria	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	0 (0%) 0	1 (2·5%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·3%) 1	0 (0%) 0

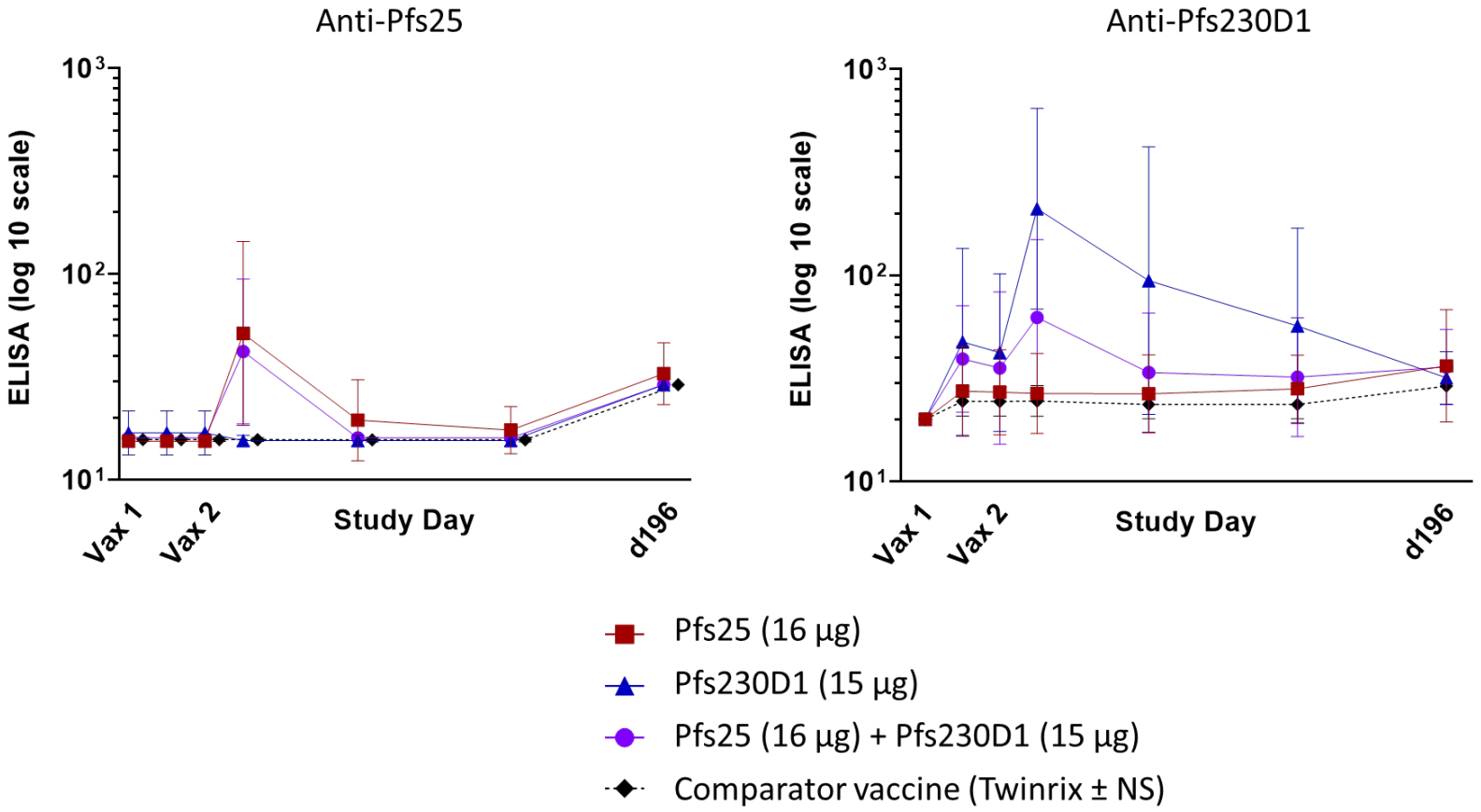
5.11 Table S10. Main cohort laboratory abnormalities

Laboratory AEs were assessed until 14 days post vaccination + visit window timeframe (+3 days). Scheduled labs (complete blood cell count with differential, alanine transaminase (ALT), creatinine) were completed on day of vaccination and then 3 and 14 days post vaccination. Laboratory adverse events were collected for the following: hemoglobin decreased, thrombocytopenia, leukocytosis, leukopenia, neutropenia, ALT increase, blood creatinine increased and for any medically important laboratory abnormality (at the discretion of the investigator). Vaccinations were administered on a 0, 1, 4·5, 16·5 month schedule from May to October 2015 (for dose 1, 2, 3) and September to October 2016 (for dose 4). All AEs were coded using MedDRA and preferred terms provided. X (XX%) X = number of unique subjects experiencing AEs (percentage of subjects with AEs) absolute number of AEs. Vax = Vaccination. AE = adverse events; µg = micrograms. No significant differences were seen.

	Pfs25, 47 µg + NS				Pfs230, 40 µg + NS				Pfs25, 47 µg + Pfs230, 40 µg				TWINRIX/Menactra + NS			
	Vax 1 (N=50)	**Vax 2 (N=48)**	**Vax 3 (N=44)**	**Vax 4 (N=42)**	**Vax 1 (N=49)**	**Vax 2 (N=45)**	**Vax 3 (N=43)**	**Vax 4 (N=40)**	**Vax 1 (N=50)**	**Vax 2 (N=50)**	**Vax 3 (N=46)**	**Vax 4 (N=39)**	**Vax 1 (N=51)**	**Vax 2 (N=47)**	**Vax 3 (N=44)**	**Vax 4 (N=40)**
Laboratory AEs	8 (16%) 8	8 (16·7%) 9	4 (9·1%) 4	0 (0%) 0	5 (10·2%) 5	8 (17·8%) 10	2 (4·7%) 2	5 (12·5%) 5	4 (8%) 4	5 (10%) 5	2 (4·3%) 2	5 (12·8%) 6	7 (13·7%) 8	6 (12·8%) 7	6 (13·6%) 7	4 (10%) 4
Grade 1	6 (12%) 6	5 (10·4%) 6	1 (2·3%) 1	0 (0%) 0	5 (10·2%) 5	7 (15·6%) 9	1 (2·3%) 1	3 (7·5%) 3	4 (8%) 4	3 (6%) 3	2 (4·3%) 2	4 (10·3%) 4	5 (9·8%) 6	6 (12·8%) 6	6 (13·6%) 7	3 (7·5%) 3
Grade 2	2 (4%) 2	3 (6·3%) 3	3 (6·8%) 3	0 (0%) 0	0 (0%) 0	1 (2·2%) 1	0 (0%) 0	2 (5%) 2	0 (0%) 0	2 (4%) 2	0 (0%) 0	2 (5·1%) 2	1 (2%) 1	1 (2·1%) 1	0 (0%) 0	1 (2·5%) 1
Grade 3	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Grade 4	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Individual Laboratory AEs																
Anemia/Hemoglobin Decreased	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	2 (5·1%) 2	0 (0%) 0	1 (2·1%) 1	1 (2·3%) 1	0 (0%) 0
Leukopenia	2 (4%) 2	2 (4·2%) 2	1 (2·3%) 1	0 (0%) 0	4 (8·2%) 4	3 (6·7%) 3	0 (0%) 0	1 (2·5%) 1	3 (6%) 3	1 (2%) 1	1 (2·2%) 1	1 (2·6%) 1	2 (3·9%) 2	2 (4·3%) 2	2 (4·5%) 2	1 (2·5%) 1
Leukocytosis	0 (0%) 0	1 (2·1%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0
Neutropenia	3 (6%) 3	3 (6·3%) 3	2 (4·5%) 2	0 (0%) 0	1 (2%) 1	4 (8·9%) 4	0 (0%) 0	2 (5%) 2	1 (2%) 1	2 (4%) 2	1 (2·2%) 1	1 (2·6%) 1	3 (5·9%) 3	4 (8·5%) 4	3 (6·8%) 3	3 (7·5%) 3
Thrombocytopenia	1 (2%) 1	1 (2·1%) 1	1 (2·3%) 1	0 (0%) 0	0 (0%) 0	1 (2·2%) 1	0 (0%) 0	2 (5%) 2	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·6%) 1	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0
Blood Creatinine Increased	0 (0%) 0	2 (4·2%) 2	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·2%) 1	2 (4·7%) 2	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	1 (2·6%) 1	2 (3·9%) 2	0 (0%) 0	1 (2·3%) 1	0 (0%) 0
ALT Increased	1 (2%) 1	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0	0 (0%) 0

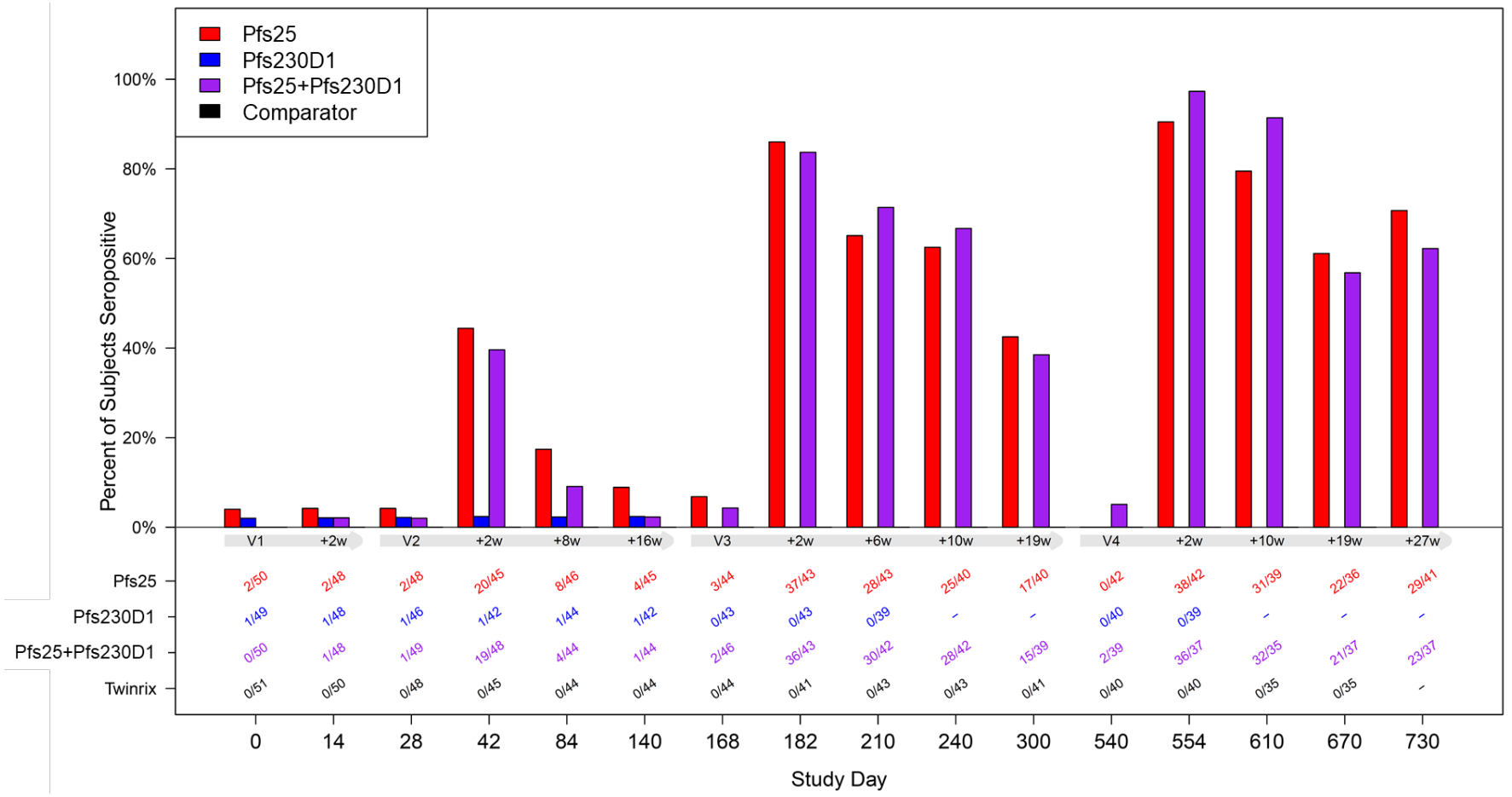
5.12 Figure S2. Pilot study: antibody titres for single and combination immunogen arms by ELISA.

Anti-Pfs25 and anti-Pfs230D1 antibody titres were determined by ELISA for each vaccination group as well as comparator. Geometric means are presented with error bars indicating 95% confidence interval. Vaccinations were administered on a 0, 1 month schedule from April to May 2015. Follow-up concluded by November 2015 (study day 196, ~6 months post dose 2). ELISA titres were evaluated at each vaccination, 2 weeks post dose 1 and 2, as well as at 8, 16, 24 weeks post dose 2.



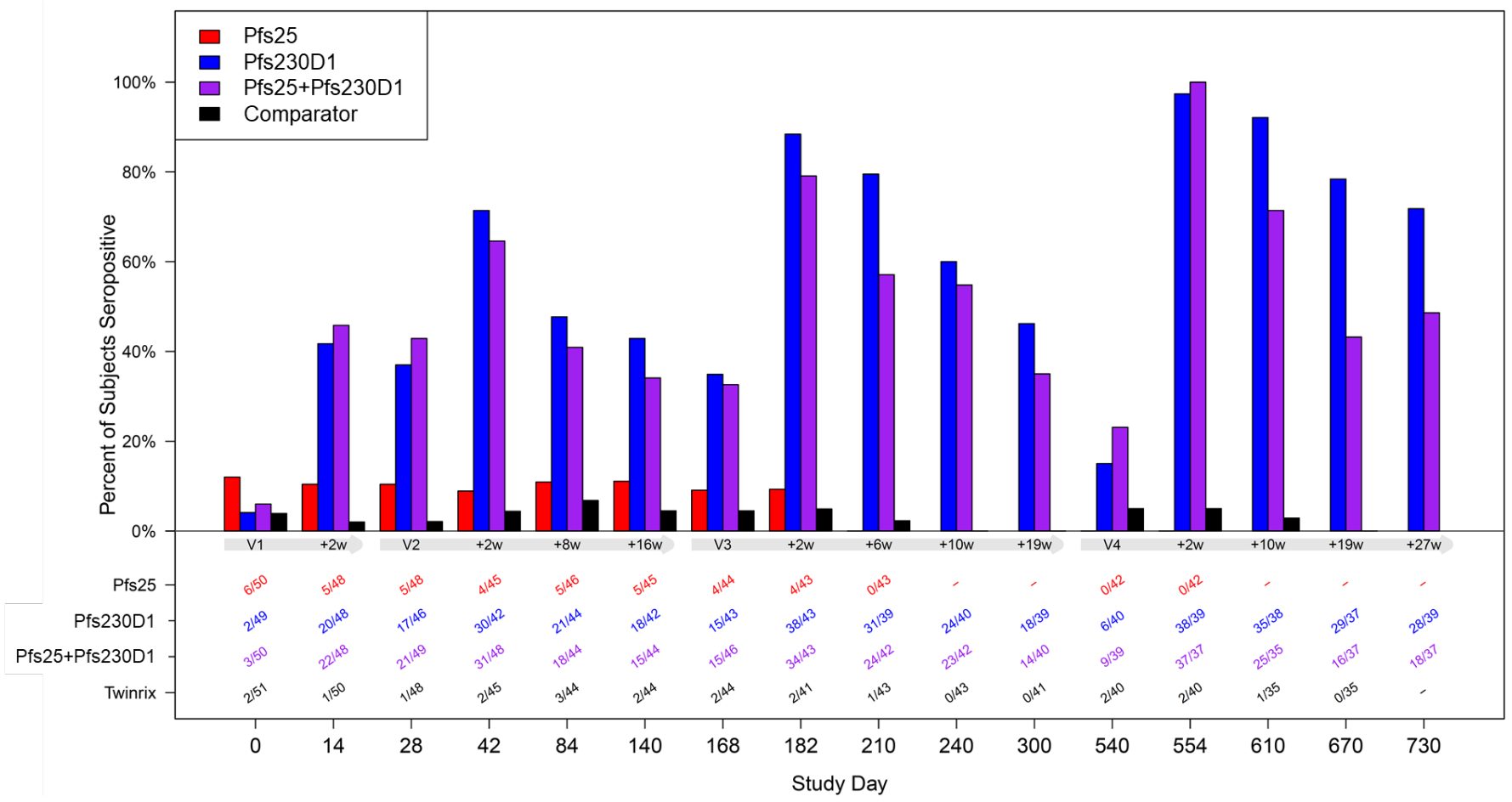
5.13 Figure S3. Proportion of Pfs25 seropositive participants by study arm.

Seropositivity was defined as greater than 3 standard deviations above the mean plate level of detection, averaged across all ELISA plates. Pfs25 = 47 µg of Pfs25-EPA/Alhydrogel® + normal saline; Pfs230D1 = 40 µg of Pfs230D1-EPA/Alhydrogel® + normal saline; Pfs25+Pfs230D1 = 47 µg of Pfs25-EPA/Alhydrogel® + 40 µg of Pfs230D1-EPA/Alhydrogel®; comparator = Twinrix (dose 1-3) or Menactra (dose 4) + normal saline



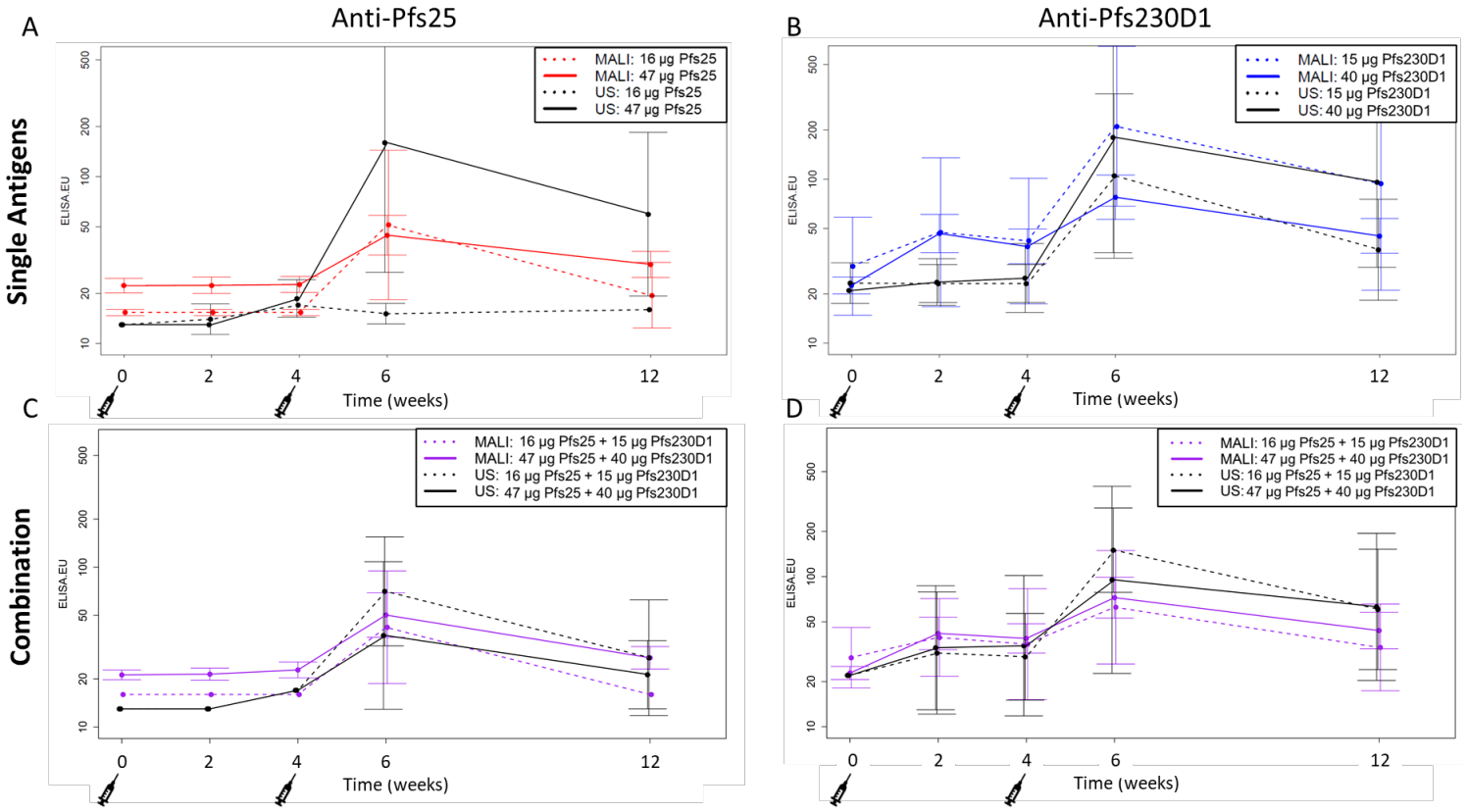
5.14 Figure S4. Proportion of Pfs230D1 seropositive participants by study arm.

Seropositivity was defined as greater than 3 standard deviations above the mean plate level of detection, averaged across all ELISA plates. Pfs25 = 47 µg of Pfs25-EPA/Alhydrogel® + normal saline; Pfs230D1 = 40 µg of Pfs230D1-EPA/Alhydrogel® + normal saline; Pfs25+Pfs230D1 = 47 µg of Pfs25-EPA/Alhydrogel® + 40 µg of Pfs230D1-EPA/Alhydrogel®; comparator = Twinrix (dose 1-3) or Menactra (dose 4) + normal saline



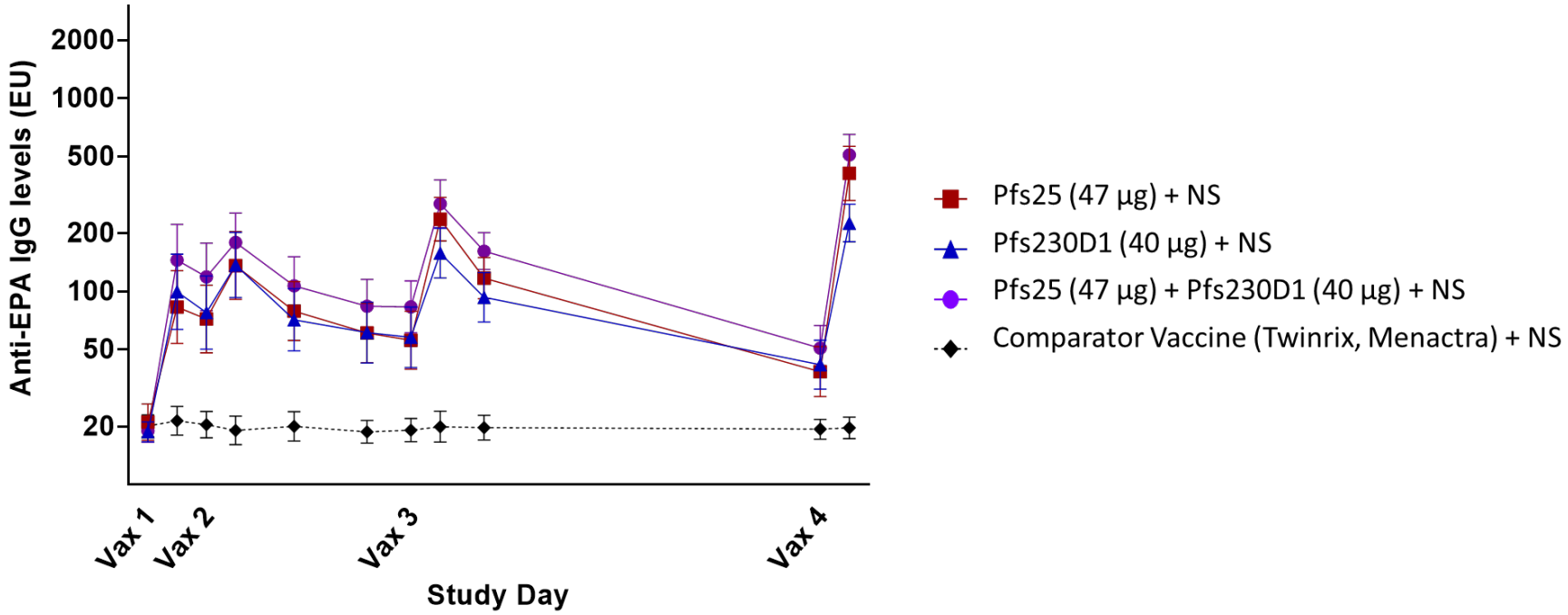
5.15 Figure S5. TBV antibody responses in Mali versus US populations

Post-hoc analysis: vaccinations were administered on a 0, 1 month schedule for all subjects shown. All US subjects (low, Pfs25 = 16µg, Pfs230D1 = 15µg; high dose, Pfs25 = 47µg, Pfs230D1 = 40µg) only received two vaccinations. Mali pilot (low dose; Pfs25 = 16µg, Pfs230D1 = 15µg) only received two vaccinations. Mali main (high dose, Pfs25 = 47µg, Pfs230D1 = 40µg) received doses on a 0, 1, 4·5, 16·5 month schedule, but below is only shown through 3 months post dose 2 (prior to receipt of dose 3 or 4) for comparison. Dotted lines = low dose arms; solid lines = high dose arms. Black = US subjects; Red = Mali subjects receiving Pfs25 containing regimens; Blue = Mali subjects received Pfs230D1 containing regimens. Geometric means are presented with error bars indicating 95% confidence interval.



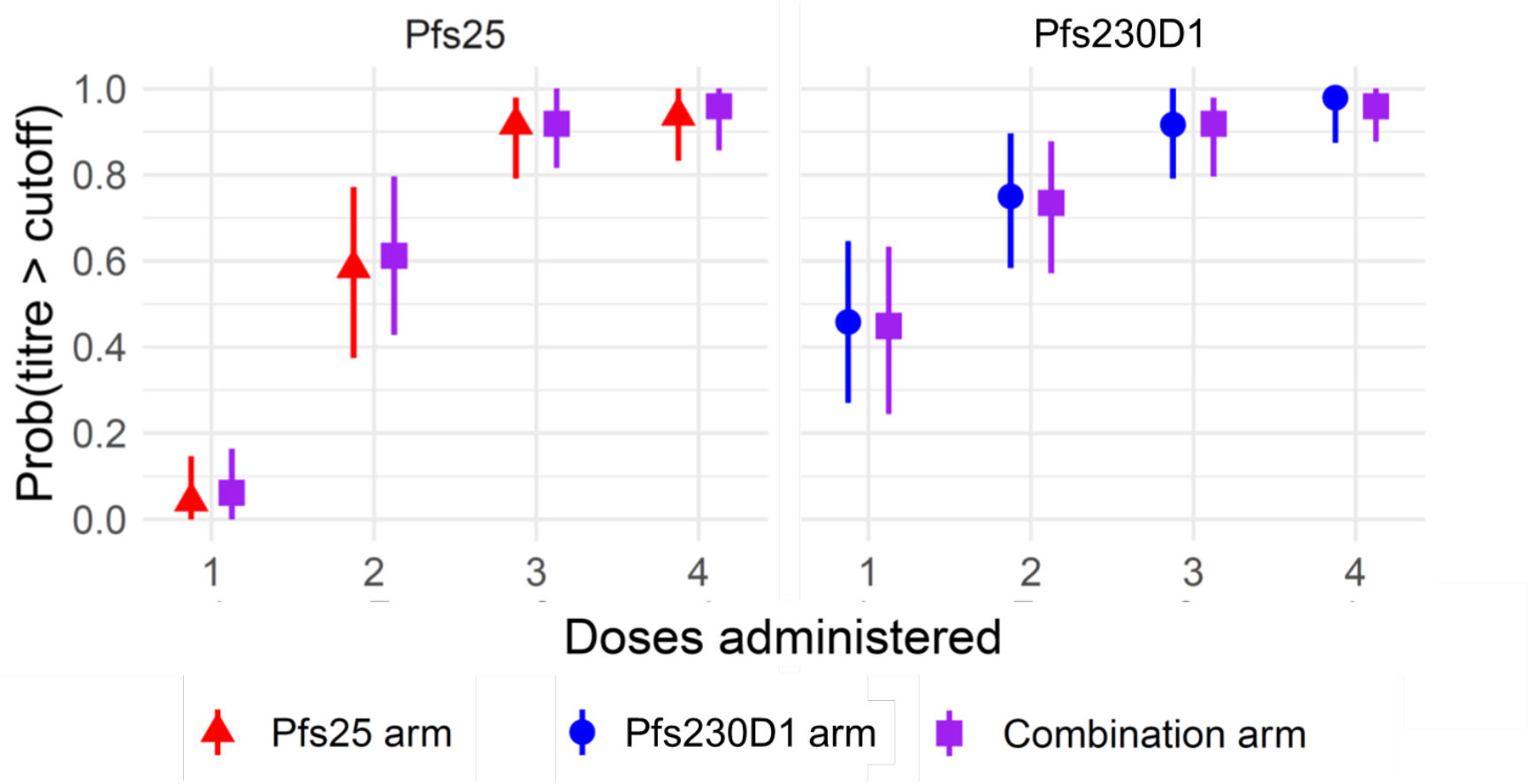
5.16 Figure S6. Anti-EPA titres were consistent among vaccinated groups.

Anti-EPA antibody titres were determined by ELISA for each vaccination group as well as comparator. Geometric means are presented with error bars indicating 95% confidence interval. Main cohort participants received vaccinations on a 0, 1, 4·5, 16·5-month schedule. ELISA sampling timepoints post vaccination can be seen in **Table S4B**. NS = normal saline.



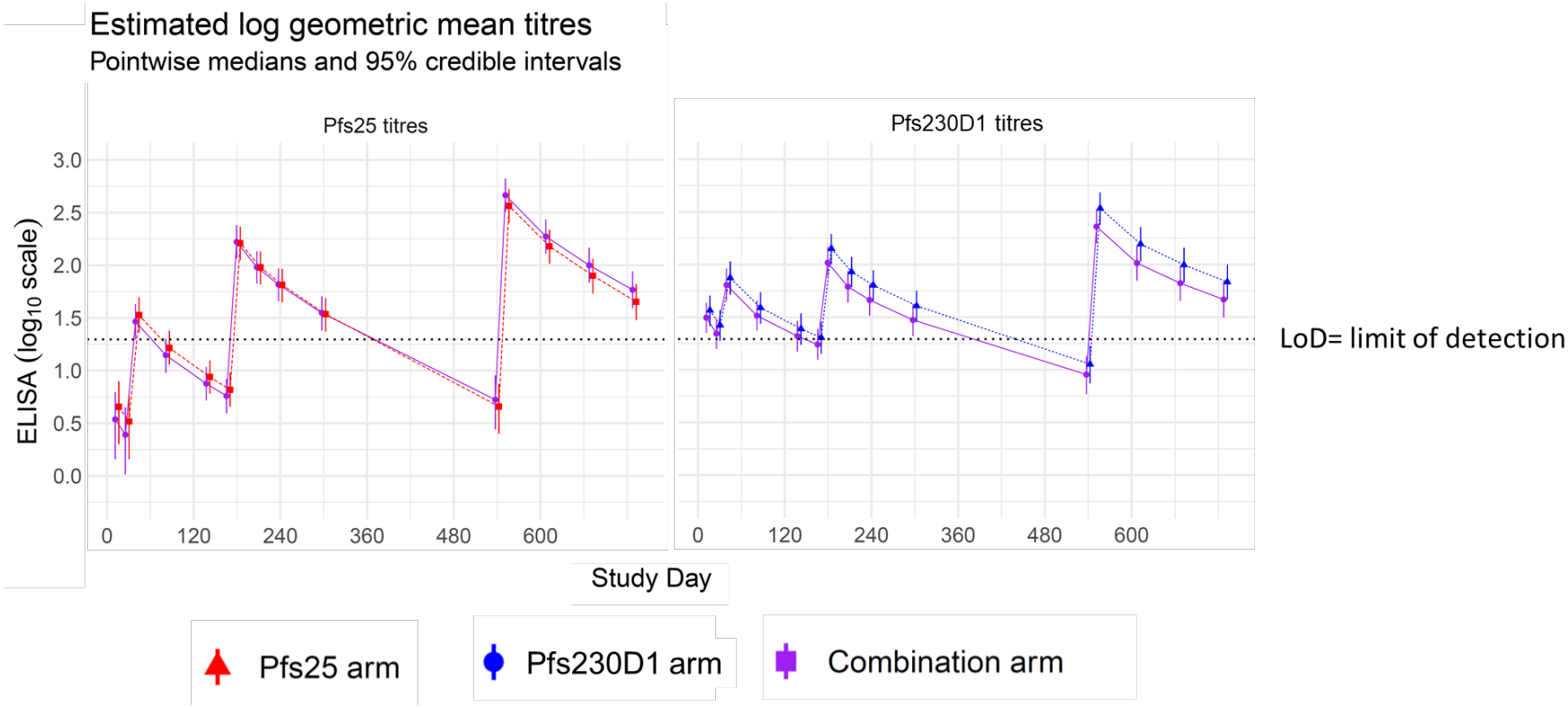
5.17 Figure S7. Probability of antibody response by number of doses administered.

Probability of antibody responses was examined by Bayesian proportional odds logistic regression model.



5.18 **Figure S8. Bayesian model of antibody profiles.**

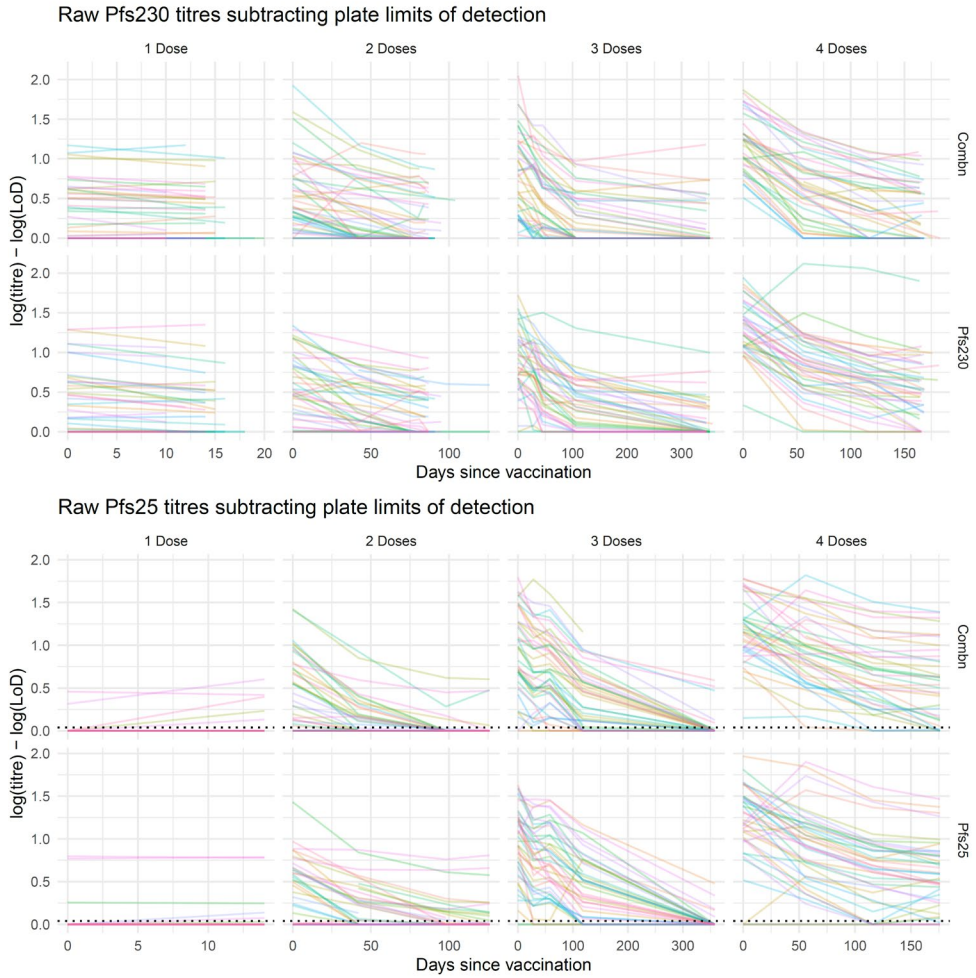
Log geometric mean antibody titres by arm (curves with point estimates and credible intervals overlayed), and average plate limits of detection for each antigen (dashed lines).



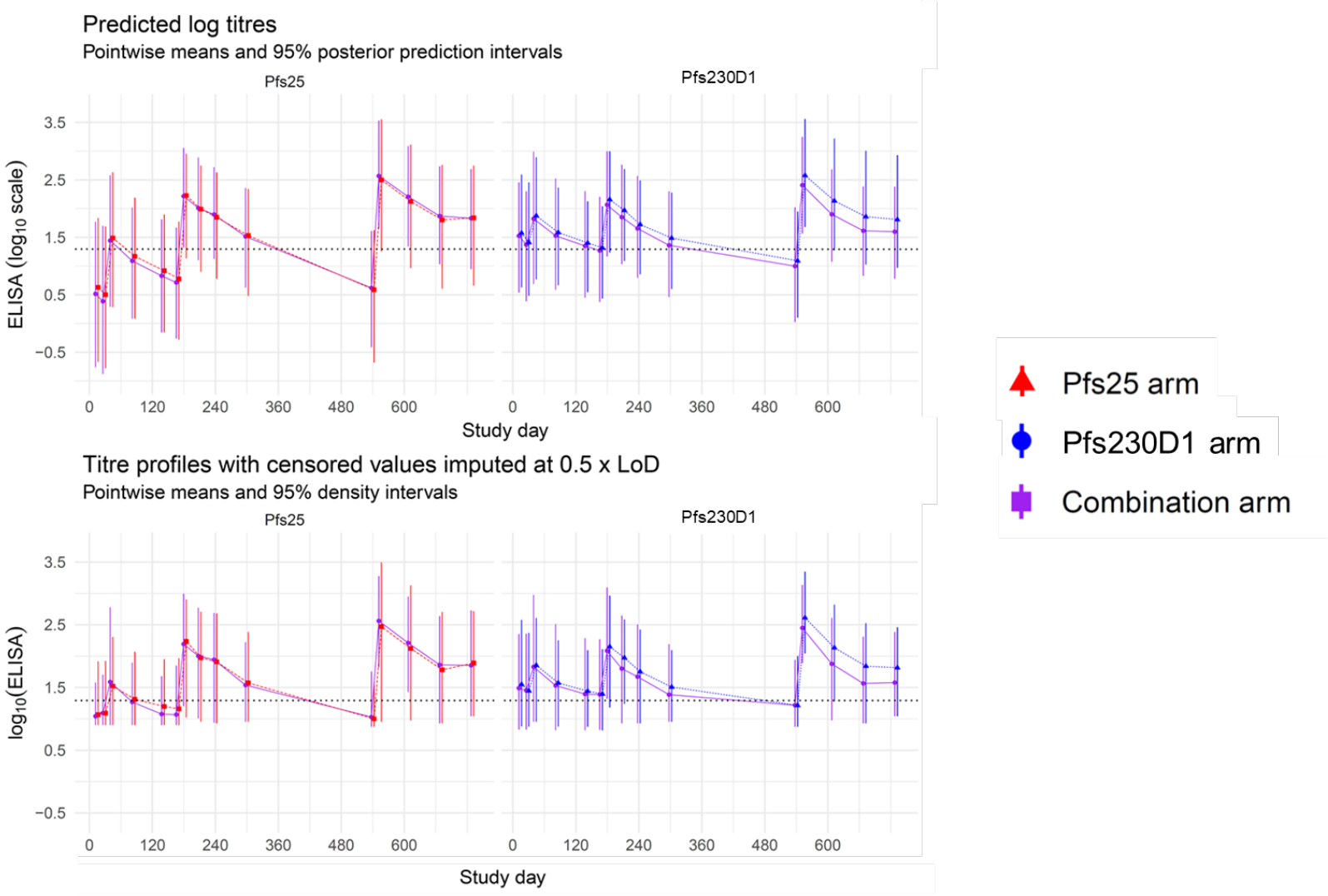
5.19 Figure S9. Estimated antibody decay profiles

5.20 **Figure S10. Individual antibody titre measurements after subtracting ELISA plate limits-of-detection.**

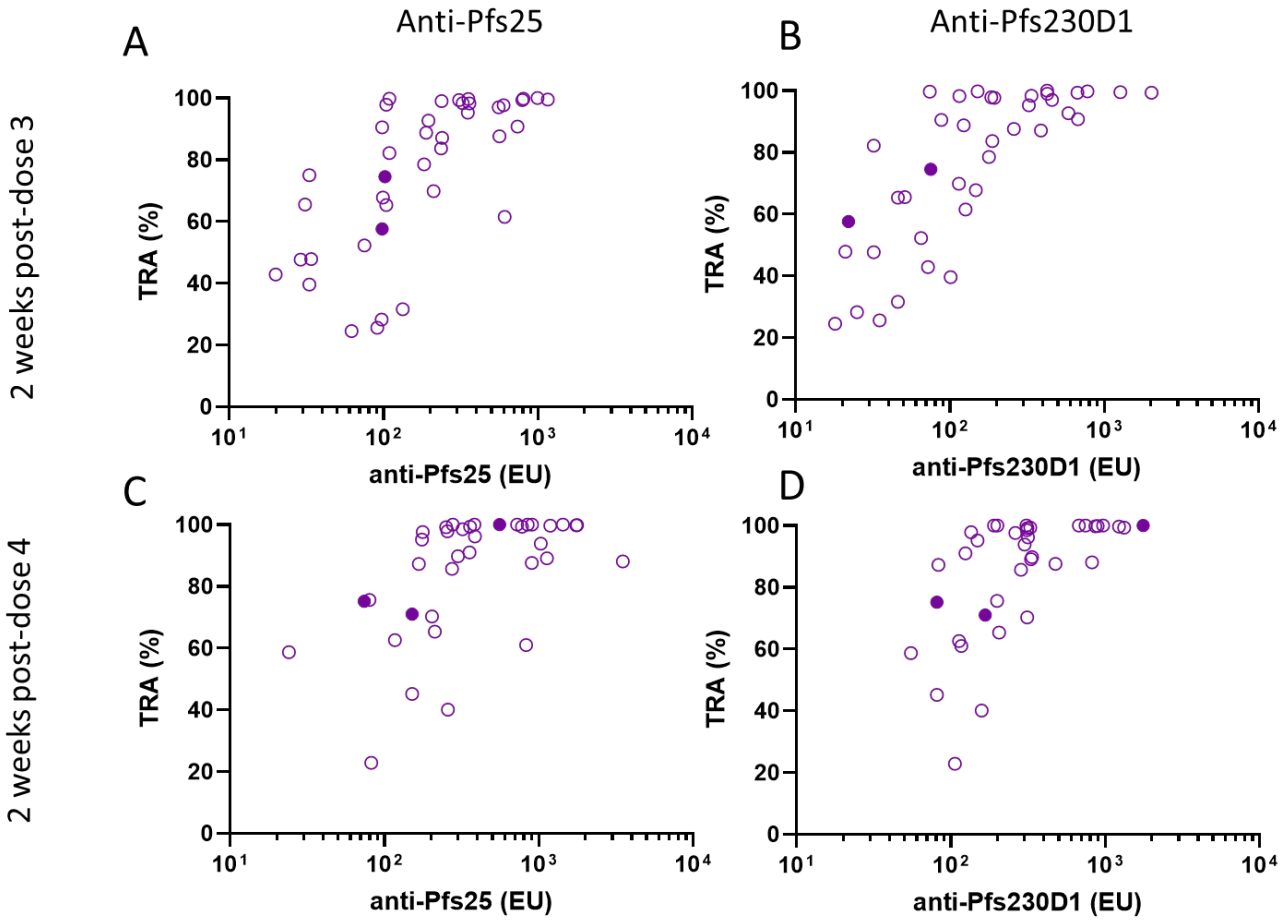
Each line represents one participant.



5.21 Figure S11. Antibody decay model predictions



5.22 Figure S12. Transmission reducing activity is associated with antibody titre for Pfs25+Pfs230D1 combination vaccine.

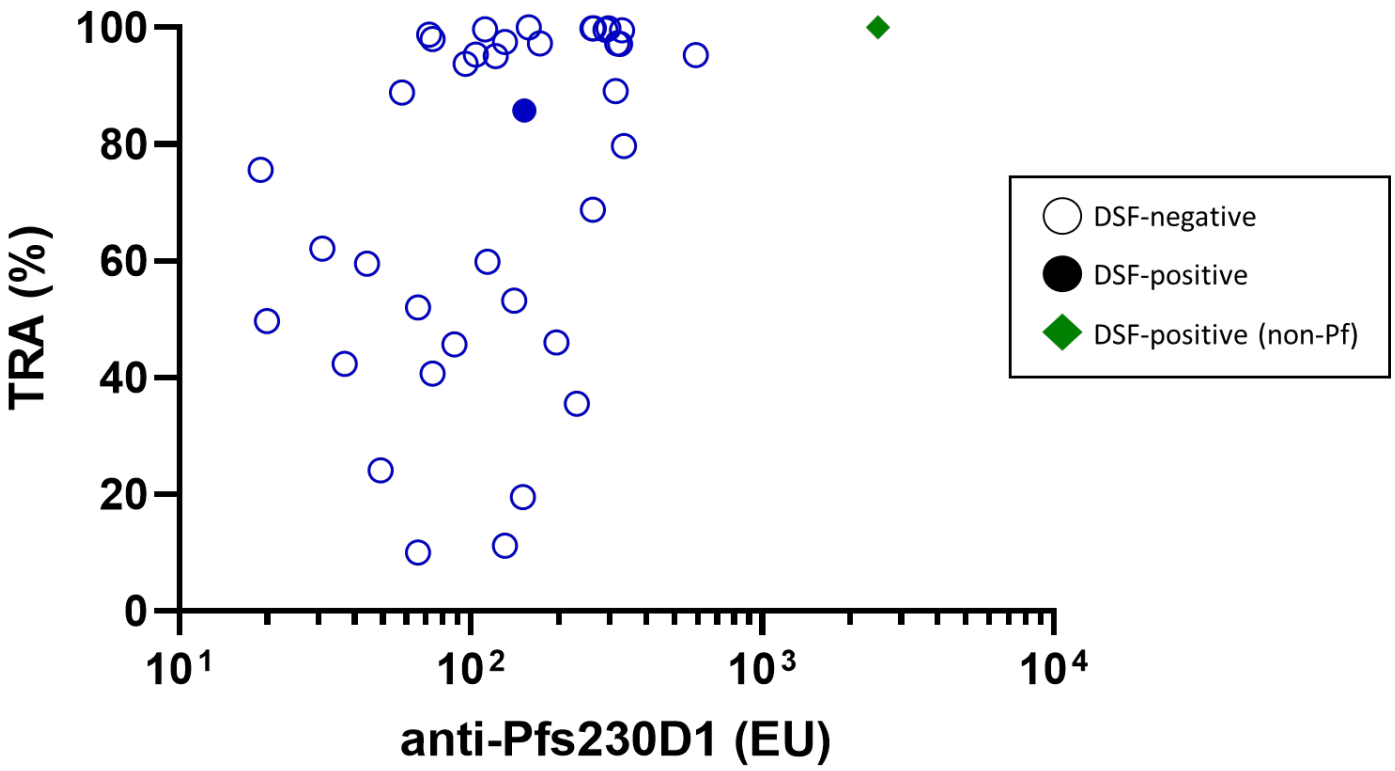


E

Linear regression analysis

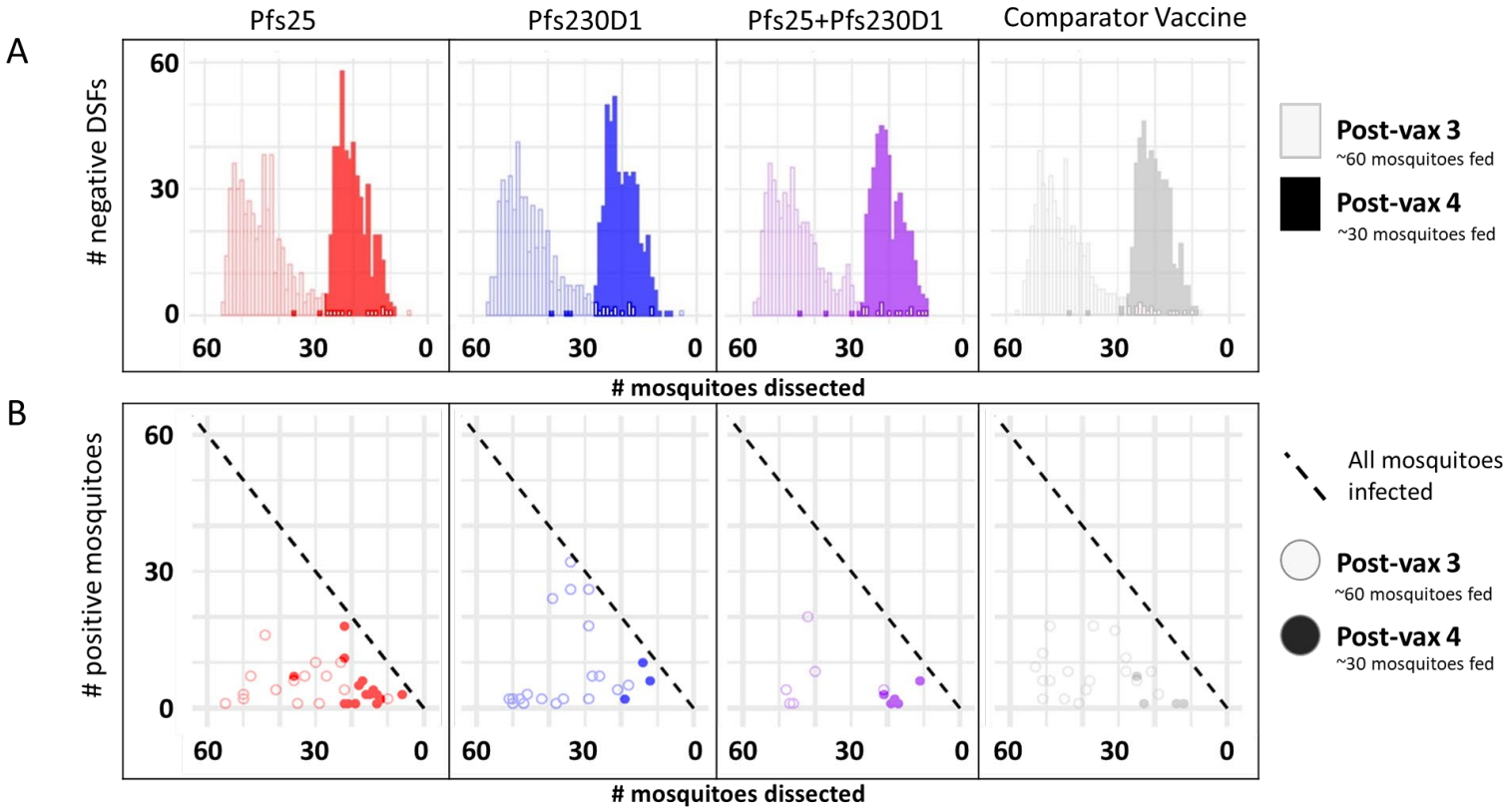
Vaccine arm	Test Antigen	ELISA value to achieve 80% TRA
Pfs25 + Pfs230D1	Pfs25	298
Pfs25 + Pfs230D1	Pfs230	223

5.23 **Figure S13. Transmission reducing activity is associated with Pfs230D1 antibody titre 10 weeks post-dose 4.**



5.24 **Figure S14. Direct skin feeding assay.**

The top row pertains to negative (all mosquitoes are uninfected) DSFs while the bottom row pertains to the positive DSFs. Top row: Only all-zero (negative) DSF data are displayed. Histograms are plotted where the x-axis is number of mosquitoes dissected in a DSF while the y-axis is the count of how many DSFs were negative at each level of number mosquitoes dissected. Bottom row: Only positive DSF data are displayed. The x-axis is number of dissected mosquitoes in the DSF while the y-axis is the number of positive mosquitoes in the DSF. A dot on the dashed line represents a positive DSF where every mosquito was infected.



5.25 Table S11. DSF Group Summary

	Year 1 (2015)[A]					Year 2 (2016)[B]					TOTALS
	Pfs25 + normal saline	**Pfs230D1 + normal saline**	**Pfs25 + Pfs230D1**	**TWINRIX + normal saline**	**TOTAL**	**Pfs25 + normal saline**	**Pfs230D1 + normal saline**	**Pfs25 + Pfs230D1**	**Menactra + normal saline**	**TOTAL**	
N. DSF Completed	502	491	504	510	2007	480	463	436	464	1843	3850
N. Subjects Completing ≥1 DSF	44	43	44	44	175	41	40	37	40	158	333
N. Positive DSFs	15	19	6	18	58	18	3	5	4	30	88
N. Positive DSF Subjects	4	6	2	6	18	9[B]	2	3	4	18	36
N. Positive Mosquitoes[C]	81	167	38	145	431	73	18	13	10	114	545
Avg. Oocyst count (range); Positive Feeds only	2·86 (1-20)	10·93 (1-99[D])	2·03 (1-9)	2·49 (1-13)	5·39 (1-99[D])	2·86 (1-27)	2·52 (1-5)	1·67 (1-6)	1·50 (1-7)	2·42 (1-27)	3·91 (1-99)
Feeding Rate	97·8	97·4	97·3	97·4	97·5	94·2	94·4	94·6	94·7	94·4	96·0
Survival Rate	79·0	79·1	79·8	79·2	79·3	78·9	78·6	78·5	78·8	78·7	79·0

[A]DSF were conducted using only 15 mosquitoes per cup (30 total for feeds in 2016 vs 60 total for feeds conducted in 2015)

[B]One individual was positive in both primary and booster series; 8 unique individuals in the study as a whole were positive in Pfs25 arm in the booster season

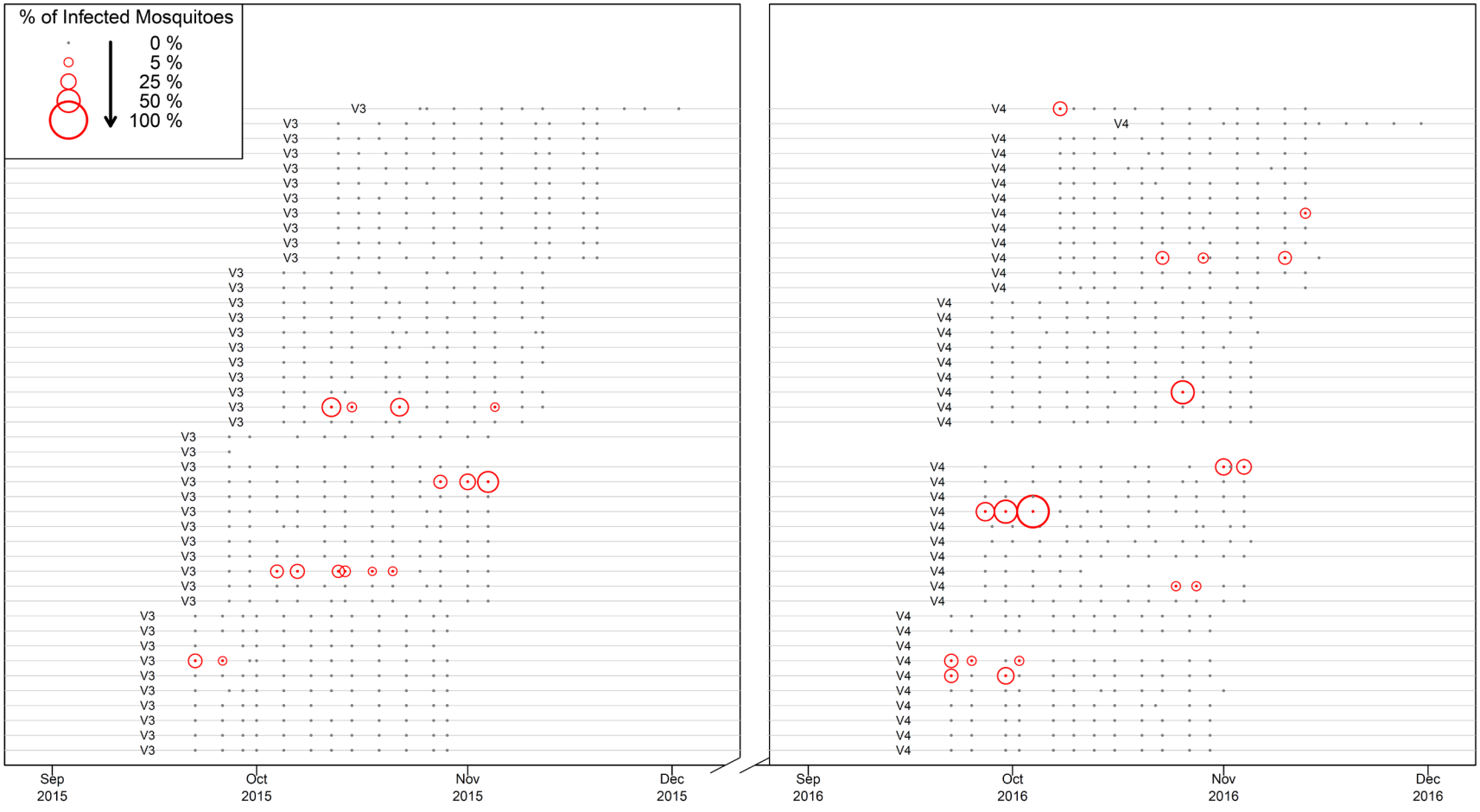
[C]Positive mosquitoes here includes all species of *Plasmodium.* A single infected midgut was analyzed for *Plasmodium* species in 63 out of 88 infected feeds. From these 63 midguts, 46 speciation results were obtained including 44 *P. falciparum*-infected midguts and 2 *P. ovale*-infected midguts

[D]Max oocyst counted is 99, above is marked as >99; 99 used for calculations

5.26 Figure S15. DSF Individual Summary.

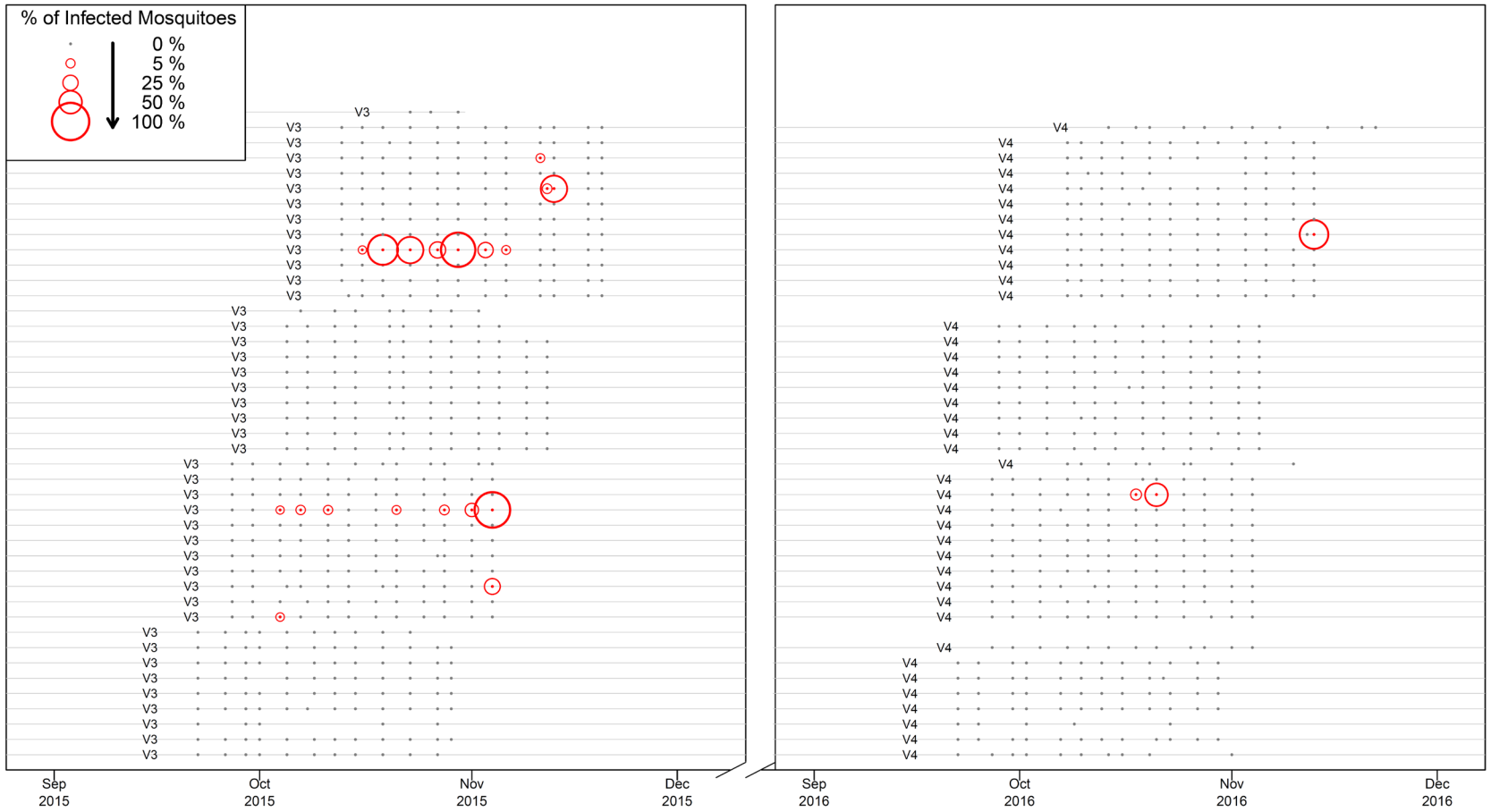
Each subject is depicted by timelines over two seasons that indicate DSF timepoints and their outcomes, stratified by trial arm. Small dots are negative DSFs. Large square and diamond shapes are positive DSFs. Dot and shape color conveys peripheral blood asexual parasites detected at time of DSF by blood smear (red for falciparum, green for ovale, black for no asexual parasites). Square or diamond shape denotes the presence or absence of ~~sexual~~ gametocytes by blood smear at time of DSF, with diamonds denoting falciparum gametocytes and squares denoting absence of detected gametocytes. Large circles surrounding positive DSF shapes show oocyst speciation results 7 days after feed, with color conveying the oocyte species call (red for falciparum, green for ovale). For example, a red diamond inside a red circle denotes a feed visit where the peripheral smear was positive for both asexual and gametocyte falciparum parasites, and the DSF resulted in falciparum speciated oocysts. While a black square inside a red circle denotes a feed visit that detected no asexual or gametocyte parasites by peripheral blood smear, but nonetheless had a positive DSF result with falciparum speciated oocysts.

Pfs25 (47 µg) + Normal Saline



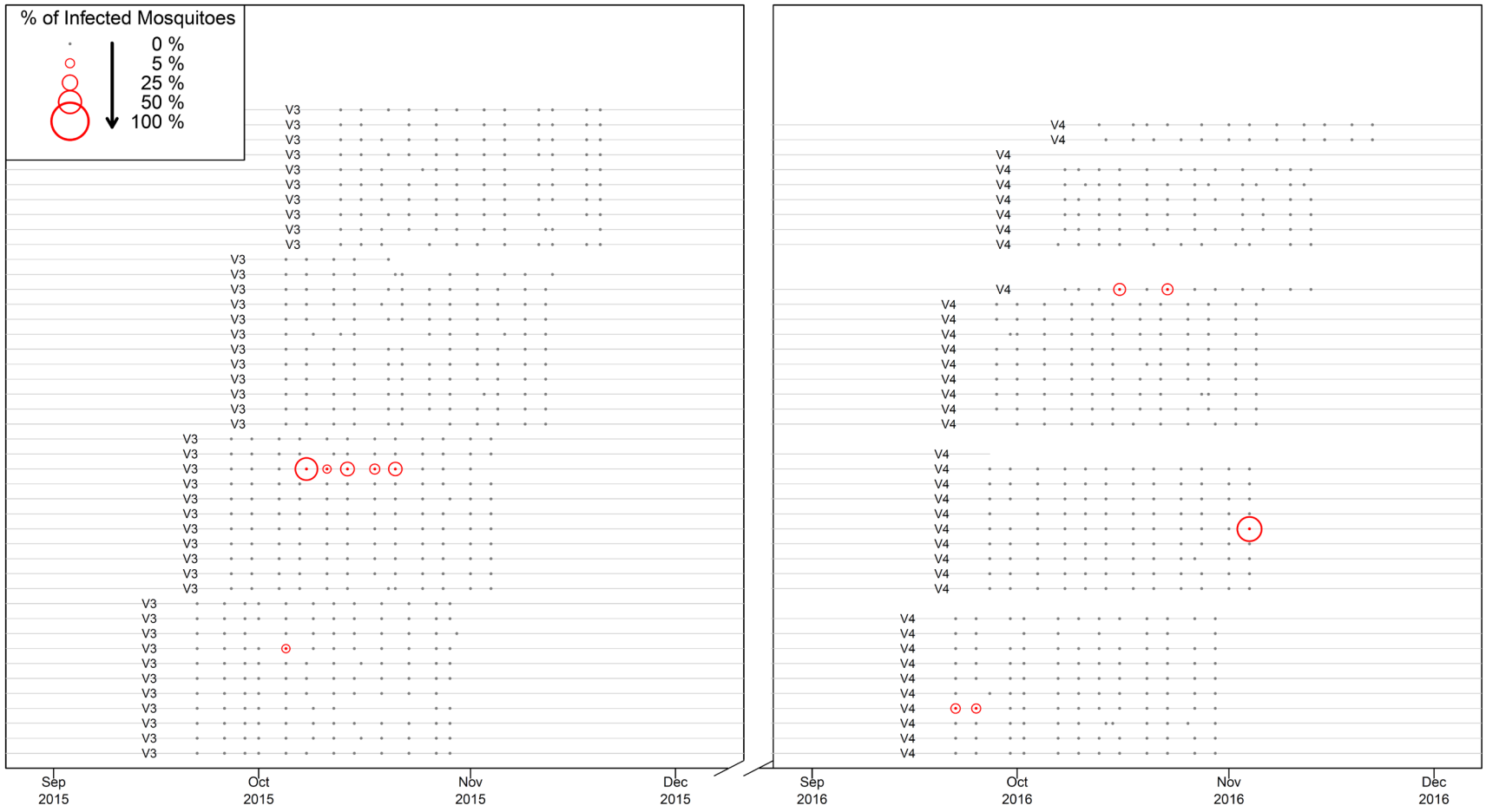
1466

Pfs230D1 (40 µg) + Normal Saline



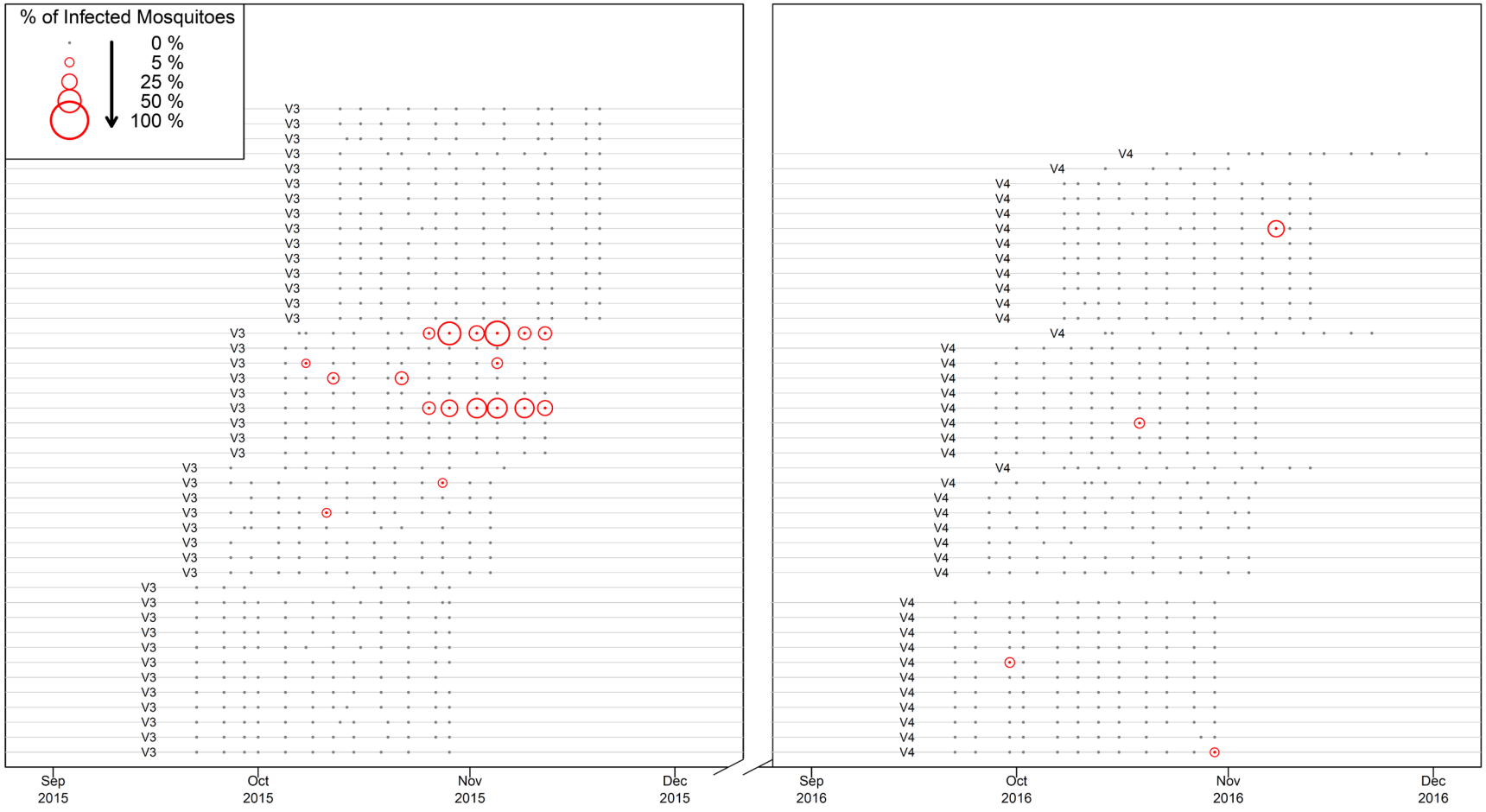
1467

Pfs25 (47 µg) + Pfs230D1 (40 µg)



1473

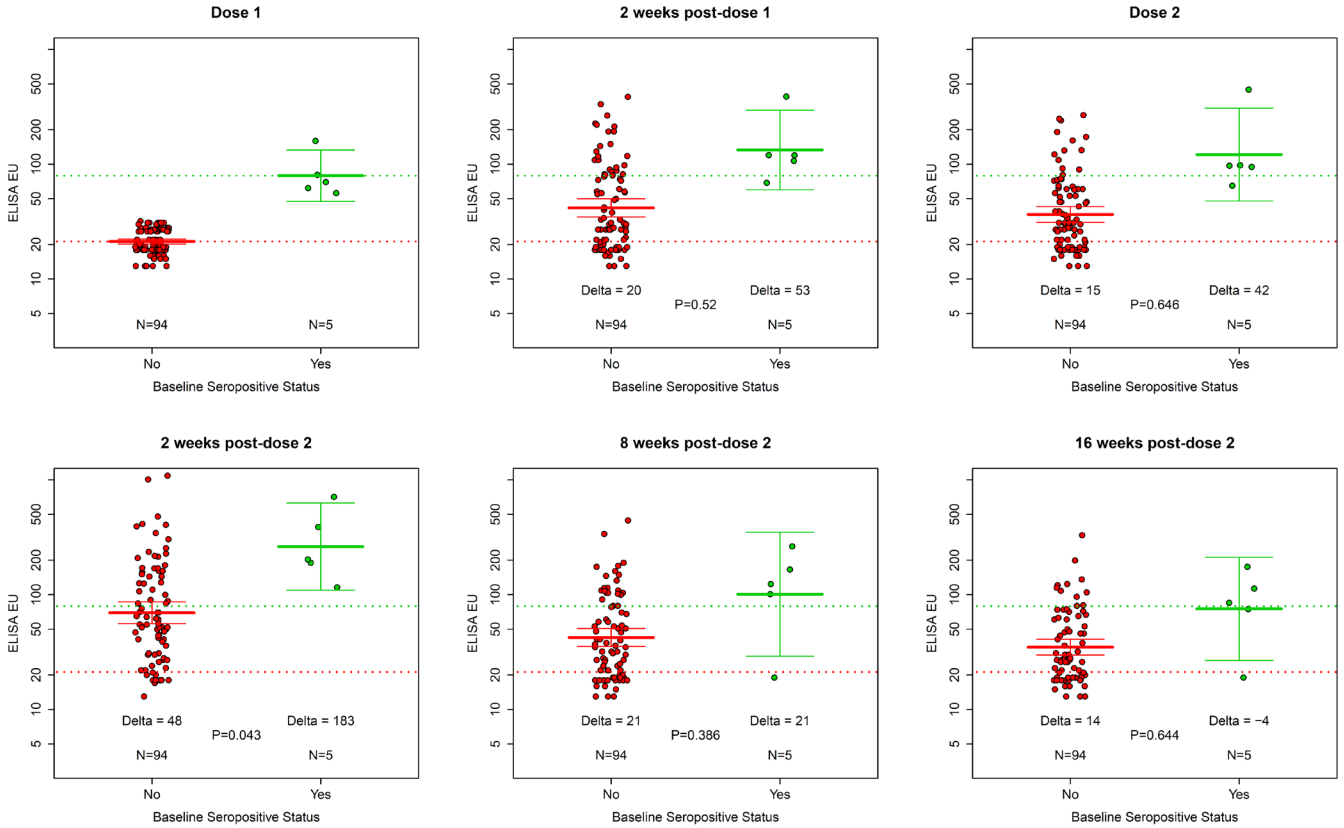
TWINRIX /Menactra + NS



1474

5.27 Figure S16. Pfs230D1-specific antibody responses in participants who received Pfs230D1-EPA alone or in combination with Pfs25-EPA, stratified by Pfs230D1 baseline seropositivity.

We compared 5 participants who were Pfs230D1-seropositive at baseline (2 in the Pfs230D1-EPA alone group; 3 in the Pfs230D1-EPA+Pfs25-EPA combination group) against 94 participants who were not seropositive (47 and 47, respectively). Vaccines were administered on Days 0, 28, 56. Shown are boxplots for actual ELISA units at each timepoint. Dotted horizontal lines represent mean EU value for each group at baseline. The delta EU for each timepoint compared to baseline is indicated below each boxplot; p-values indicate significant differences between groups for their delta values (change from baseline).



6 REFERENCES

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