**Statistical Analysis Plan**

**Investigation of *Plasmodium vivax* Parasitaemia and Fever**

The *P. vivax* Fever Study Group

**Version 1.1**

**Suggested citation:** Statistical Analysis Plan, The *P. vivax* Fever Study Group: Investigation of *Plasmodium vivax* Parasitaemia and Fever.

**Version History**

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| Version number | Revision(s) and reason(s) for amendment | Release date |
| v1.0 |  | 18/11/2021 |
| v1.1 | * Age groups changed from 4 to 3 groups (there were too few patients in one group)
* Outcomes analysed by relapse periodicity rather than transmission intensity (a more accurate reflection of endemicity for vivax malaria)
* Primary outcome measure for Objective 1 changed from 5th to 10th centile of parasitaemia (less influenced by outlying values)
* Update to the writing group authors
 | 14/02/2022 |

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**2. Introduction and Rationale**

*Plasmodium vivax* malaria is an important threat to public health in many countries across the Asia-Pacific, Horn of Africa, and the Americas1,2. It has been increasingly associated with severe and fatal outcomes, and repeated hypnozoite-induced relapses cause a cumulative risk of anaemia and malnutrition which results in substantial indirect morbidity and mortality3,4.

When a mosquito infected with a *Plasmodium* species takes a blood meal from a human, it releases sporozoites into the human circulation, which infect liver cells, incubating for ~5 to 21 days before rupturing and releasing merozoites into the peripheral bloodstream. In the case of *P. vivax* or *P. ovale*, parasites can lie dormant in the liver (hypnozoites), “reactivating” weeks to months after the initial infection to cause recurrent episodes of malaria (relapses). Following rupture of a parasitised hepatocyte, approximately 104 merozoites are released into the peripheral circulation, subsequently undergoing asexual reproduction every 48 hours. In non-immune individuals, the peripheral parasite count rises exponentially, with symptoms associated with the innate immune response beginning to occur at about the level of microscopic detection (~50/µL); in an adult this equates to a total infection of approximately 108 parasites5,6. However, repeated episodes of malaria can result in the host developing pathogen-specific immunity, allowing some individuals to harbor higher parasitaemias without manifesting symptoms.

The pyrogenic threshold of a *Plasmodium* infection is defined as the parasite density that is required to induce a fever. Pyrogenic thresholds vary according to parasite species and strain, and are dynamic within and between individuals according to age, immunity, and background levels of endemicity in a community.

In malaria-endemic countries, an understanding of the relationship between fever and *P. vivax* parasitaemia can inform both clinical management and epidemiological studies of disease burden. However, priorities differ in these two settings. In a clinical context, the priority is to identify as many patients as possible with malaria (by correctly attributing fever to parasitaemia). In an epidemiological context it is more helpful to understand the pyrogenic threshold and how this varies in different populations and locations.

We propose an individual patient data (IPD) meta-analysis of patients with *P. vivax* parasitaemia enrolled intoprospective clinical efficacy studies to explore both priorities. Antimalarial efficacy studies of patients with *P. vivax* malaria will be identified from a systematic review conducted previously (please see Section 4 for further details).

The limits of detection of currently available vivax RDTs make them suitable diagnostic tests for identifying patients with symptomatic parasitaemia (clinical malaria), but not asymptomatic, low-level parasitaemia.

In Objective 1 the *P. vivax* parasitaemia threshold that would capture certain percentages of cases of symptomatic malaria will be determined, to inform suitable definitions of target thresholds for RDTs – i.e. the lowest parasite density that RDTs need to detect, therefore informing RDT Target Product Profile.

In Objective 2 the pyrogenic density of *P. vivax* in the first recurrent infection will be determined and its variation with age and endemicity explored. In addition, other key determinants of fever at recurrence will be identified. Patients who experience recurrent *P. vivax* parasitaemia during follow up in clinical trials comprise a patient population who may or may not have a fever or history of fever at the time of clinical review. In this context it is possible to compare febrile, parasitaemic patients with afebrile, parasitaemic patients to estimate the *P. vivax* pyrogenic threshold, in contrast to the initial presentation and enrolment into the trial during which the vast majority of patients will have experienced fever or other symptoms of malaria.

Since the majority of *P. vivax* malaria and transmission occur as a result of recurrences7, the analysis of recurrent episodes will allow pyrogenic density to be estimated and provide an opportunity to investigate how pyrogenic density varies with successive recurrences.

**3. Aims**

* 1. To inform the development and evaluation of diagnostic tools for vivax malaria by estimating the parasitaemia thresholds which capture certain percentages of febrile patients who present for treatment.
	2. To determine the pyrogenic density of *P. vivax* parasitaemia in patients with recurrent infection, and how this varies with age, location, and first versus second recurrence.

Aim 3.1 will be achieved by analysing febrile patients at enrolment into clinical trials or subsequently presenting with febrile recurrences. Aim 3.2 will focus on patients in clinical trials actively followed after initial treatment, re-presenting with parasitaemia with and without fever.

**4. Study Identification and Data Pooling**

Prospective clinical efficacy studies of *P. vivax* mono-infection were identified from a systematic review conducted previously and updated as of 16th February 20218. The inclusion and exclusion criteria of this systematic review are listed below:

**Inclusion criteria:**

Prospective clinical efficacy studies of uncomplicated *P. vivax* mono-infection with a minimum of 28 days follow up

Published between January 1st 2000 and 16th February 2021

**Exclusion criteria:**

Studies on prevention or prophylaxis

Reviews

Animal studies

Patients with severe malaria

First dose of schizontocidal treatment per day unsupervised

Data extracted retrospectively from medical records

Studies of pregnant women

The essential inclusion criteria for the current study (see Section 5: Study Enrolment) were then applied to identify all studies suitable for inclusion in this analysis. All identified studies were held in the WWARN (Worldwide Anti-malarial Resistance Network) repository. A Data Access Request was granted by WWARN and investigator permission was sought and granted for each dataset included in this study. Relevant data will be curated, standardised, and pooled into a single database of quality-assured individual patient data to facilitate meta-analysis.

**5. Study Enrolment**

* 1. **Essential Inclusion Criteria**
* Prospective clinical efficacy studies of *P. vivax* mono-infection
* Study meta-data including design, inclusion and exclusion criteria, and study location
* Date of enrolment
* Age and sex of the participant
* Number of recurrent episodes of *P. vivax* parasitaemia per participant
* For each initial and recurrent episode of *P. vivax* parasitaemia:
	+ Date or timing of parasitaemic episode in relation to the date of enrolment
	+ Temperature recording and/or the presence or absence of a recent history of fever (within the last 72 hours)
	+ Presence and asexual density of *P. vivax* parasites based on blood film microscopy
	1. **Desirable Inclusion Criteria**
* Study site details
* Microscopy quality assurance information
* Residence in the study location during the last year
* Malaria history of the participant in the last year
* Weight and height of the participant
* Nutritional status of the participant, assessed using BMI (body mass index), weight-for-age z score9, or mid-upper arm circumference (MUAC)
* Haemoglobin at enrolment
* Haemoglobin at the time of recurrent *P. vivax* parasitaemia
* Schizontocidal treatment(s) tested
* Administration of schizontocidal treatment for asymptomatic recurrences
* 8-aminoquinoline (8-AQ) treatment administered and timing of administration
	1. **Exclusion Criteria**
* Pregnancy

**6. Outline of Statistical Analysis**

**6.1 Specific Objectives of the Study**

1. To inform the development and evaluation of diagnostic tools for vivax malaria:
	1. To estimate the *P. vivax* parasitaemia thresholds which capture percentages of febrile patients who present for treatment in initial infections: overall, in different age groups, and in different endemic regions
	2. To estimate the *P. vivax* parasitaemia thresholds which capture percentages of febrile patients at first recurrence: overall, in different age groups, different endemic regions, and compared to the second episode of recurrent parasitaemia
2. To inform clinical and epidemiological understanding of the relationship between fever and *P. vivax* parasitaemia:
	1. To estimate the pyrogenic density of *P. vivax* at the first recurrent infection, overall, in different age groups, different endemic regions, and compared to the second episode of recurrent parasitaemia
	2. To investigate which variables of interest are key determinants of fever and/or a recent history of fever at the first *P. vivax* recurrence

**6.2 Study Endpoints**

**Definitions:**

*Fever* will be defined as a measured axillary temperature ≥37.5°C or tympanic, oral, or rectal temperature ≥38.0°C.

*Recent history of fever* will be defined as participant-reported fever within the preceding 72 hours.

**Study Endpoints:**

Objective 1:

Peripheral asexual parasitaemia as measured by microscopy.

Objective 2:

The presence of a fever and/or a recent history of fever.

**6.3 Summary of Statistical Analyses**

**6.3.1 Summary Study Profiles**

A summary study profile of the prospective clinical efficacy studies will be presented to highlight potential selection bias. This will include (but not be restricted to):

* Country
* Region
* Estimated regional transmission intensity or parasite prevalence10/relapse periodicity11
* Year
* Study population e.g. age, sex
* Microscopy quality assurance information available
* Schizontocidal treatment administered
* 8-aminoquinoline treatment administered
* Follow-up duration
* Active and/or passive case detection during follow-up
* Proportion of recurrent episodes of parasitaemia associated with fever and/or a recent history of fever

A summary of included studies compared to potential studies will also be presented to highlight potential selection bias, and as part of the statistical analysis a sensitivity analysis will be undertaken to identify bias related to individual studies.

**6.3.2 Definitions**

*Age group* will be categorised as follows: <5 years, 5 to <15 years, and ≥15 years.

*Regional relapse periodicity* will be categorised as “high” (short) or “low” (long) according to Battle’s regions10, with high periodicity considered to include regions where the median periodicity was ≤47 days. Therefore, the regions with the two highest periodicities (region 10 and 12) where the median periodicity is ≤47 days will be categorised as “high” and others will be categorised as “low”.

The *first recurrent episode* will be defined as the first episode of recurrent *P. vivax* peripheral parasitaemia detected with microscopy after day 7 with an intervening negative smear.

The *second recurrent episode* will be defined as the second episode of recurrent *P. vivax* peripheral parasitaemia detected with microscopy after day 0, if it occurs >7 days after the first recurrent episode with an intervening negative smear.

*Transmission intensity* will be categorised as “low”, “moderate”, or “high” according to malaria endemicity estimates obtained for study regions and year from the Malaria Atlas Project10:

* Pv incidence rate ≤1 case per 1000 people per year will be categorised as a “low” transmission area;
* Pv incidence rate >1 & ≤10 cases per 1000 people per year will be classified as a “moderate” transmission area;
* Pv incidence rate >10 cases per 1000 people per year will be classified as a “high” transmission area.

*Timing of recurrence* will be categorised as follows: 8-90 days, 91-180 days, 181-270 days, or >270 days after the previous episode of *P. vivax* parasitaemia.

*Underweight* will be defined as follows:

* For children aged ≤5 years: Nutritional status will be determined using the MUAC (mid-upper arm circumference) and/or the weight-for-age z-score, using the igrowup package developed by WHO9. Those with weight-for-age or MUAC z-scores < -2 (i.e. below the 3rd centile) will be classified as underweight-for-age (termed *underweight*).
* For children and adults aged >5 years: *Underweight* will be defined as a body mass index (BMI) of <18.5.

*Anaemia* will be defined as haemoglobin <10g/dL.

*Elimination half-life of schizontocidal treatment* will be categorised as “rapid” – <1 day, “intermediate” – 1-7 days, or “slow” – >7 days. Categorisation of combination therapies will be based on the drug with the longest elimination half-life.

**6.3.3 Objective 1 – To inform the Development and Evaluation of Diagnostics for Clinical Vivax Malaria**

**Objective 1a)**

*To estimate the P. vivax parasitaemia thresholds which capture percentages of patients with fever and/or a recent history of fever who present for treatment in initial infections: overall, in different age groups, and in different endemic regions.*

Clinical efficacy studies in which no *P. vivax* parasitaemia threshold was applied for enrolment will be selected to investigate the distribution of parasitaemia in febrile patients at presentation. This distribution will be described for:

1. All eligible studies
2. Participants in each age group for all eligible studies (as defined above)
3. All eligible studies in each category of relapse periodicity (as defined above)
4. Participants in each age group for all eligible studies within each category of relapse periodicity

using the mean and standard deviation if the data are normally distributed, geometric mean and 95% reference range if the data are normally distributed following a log transformation, or the median and interquartile range if the data are non-normally distributed.

The 10th centile of *P. vivax* parasitaemia will be determined for all participants with a fever and/or a recent history of fever at presentation. In addition, subgroup analysis will be undertaken for each age group and relapse periodicity setting.

These values will correspond to the *P. vivax* parasite density above which 90% of the specified population are captured. The 1st, 5th, 25th, and 50th centiles will also be determined to demonstrate trends in the groups listed above.

A graph of percentage of “febrile” patients captured versus *P. vivax* parasitaemia will be presented, and forest plots will be constructed to explore study site effects. We anticipate that the vast majority of (if not all) patients will have been febrile or have had a recent fever at the time of enrolment and initial parasitaemia measurement. Therefore, it would not be possible to compare those with and without fever in this context.

**Objective 1b)**

*To estimate the P. vivax parasitaemia thresholds which capture percentages of patients with fever and/or a recent history of fever at first recurrence: overall, in different age groups, different endemic regions, and compared to the second episode of recurrent parasitaemia.*

The distribution of *P. vivax* parasitaemia will be described for:

1. The first and second episodes of recurrent *P. vivax* parasitaemia detected by microscopy, regardless of fever status
2. The first and second recurrent episodes where the participant had a fever and/or a recent history of fever
3. The first and second recurrent episodes where the participant did not have a fever or a recent history of fever

using the mean and standard deviation if the data are normally distributed, geometric mean and 95% reference range if the data are normally distributed following a log transformation, or the median and interquartile range if the data are non-normally distributed.

The 10th centile of *P. vivax* parasitaemia will be determined for all participants with a fever and/or a recent history of fever at first recurrence. In addition, subgroup analysis will be undertaken for each age group and relapse periodicity setting. Comparisons will also be made with the second recurrence for patients who experienced at least two recurrent episodes.

These values will correspond to the *P. vivax* parasite density above which 90% of the specified population are captured. The 1st, 5th, 25th, and 50th centiles will also be determined to demonstrate trends in the groups listed above. Exploratory analyses will be conducted to investigate how second recurrence parasitaemia distributions vary depending on the time after first recurrence.

**6.3.4 Objective 2 – To inform Clinical and Epidemiological understanding of the Relationship between Fever and *P. vivax* Parasitaemia**

**Objective 2a)**

*To estimate the pyrogenic density of P. vivax at the first recurrent infection, overall, in different age groups, different endemic regions, and compared to the second episode of recurrent parasitaemia.*

Univariable logistic regression analysis will be performed to define the relationship between the presence or absence of fever and/or a recent history of fever (dependent variable) and *P. vivax* parasitaemia at first recurrence (independent variable). A receiver operator curve (ROC) will be constructed and Youden’s index calculated to identify the parasite density value with optimal sensitivity and specificity for defining the pyrogenic threshold.

If data are only available for one of “fever” or “recent history of fever”, and the observation is negative, i.e. no measured fever or no recent history of fever, the following assumptions will be made:

1. Participants observed as no measured fever (i.e. the participant is afebrile) and with no data recorded for “recent history of fever” will be treated as having “no fever or recent history of fever”
2. Participants observed as no recent history of fever (i.e. the participant has not felt febrile in the last 72 hours) and with no available temperature measurement will also be treated as having “no fever or recent history of fever”

Subgroup analyses will be conducted using the same methodology to investigate the variation of pyrogenic density at recurrence 1 with age and relapse periodicity. For participants who had at least 2 recurrences, the pyrogenic density will be compared for recurrence 1 and recurrence 2 and subgroup analyses will be conducted to explore differences observed with timing of recurrence 2 after recurrence 1. Shared frailty analysis will also be performed to investigate study site effects.

**Objective 2b)**

*To investigate which variables of interest are predictive for fever and/or a recent history of fever at the first episode of recurrent P. vivax parasitaemia.*

Multivariable logistic regression analysis will be performed to investigate which variables of interest predict the presence or absence of fever or a recent history of fever during the first recurrent episode of peripheral *P. vivax* parasitaemia. The following variables will be investigated (see Section 6.3.2 for Definitions):

**Site**

* **Derived:**
	+ Regional transmission intensity
	+ Regional relapse periodicity
	+ Elimination half-life of schizontocidal treatment administered
	+ Timing of the first recurrence after day 0
		- This will also be explored in relation to the elimination half-life of the schizontocidal treatment administered (rapid vs. intermediate vs. slow elimination)

**Participant**

* **Baseline:**
	+ Sex
	+ Asexual parasite density
* **Derived:**
	+ Age group

**6.3.5 Additional Sensitivity Analyses**

To explore the variation in effects, sensitivity analyses will be carried out to assess the heterogeneity of studies by removing one study site at a time, and calculations of the coefficient of variation (CV) around the parameter estimates will be presented. This will be performed for the four main analyses:

1. The *P. vivax* parasitaemia capture thresholds for all participants with a fever and/or a recent history of fever at study enrolment
2. The *P. vivax* parasitaemia capture thresholds for all participants with a fever and/or a recent history of fever at first recurrence
3. The pyrogenic density of *P. vivax* at first recurrence
4. Multivariable logistic regression analysis to identify variables of interest predictive for fever and/or a recent history of fever at first recurrence

**7. Tools**

All statistical analyses will be carried out using Stata version 17.0. However, when equivalent statistical methods are applied, changing the use of statistical software does not require amendment of this SAP.

**8. Study Group Governance, Management, Co-ordination, and Publication Policy**

The *P. vivax* Fever Study Group comprises participating investigators who contribute relevant data sets to the pooled analysis. Data sets will remain the property of the investigator and will not be shared without their consent.

The Study Group will assign a Writing Committee to coordinate activities including data analysis and drafting of publications and reports for complete group review. The Writing Committee will comprise Emily Groves, Julie Simpson, Ric Price, Rob Commons, and any other participating investigators interested in undertaking the data analysis and preparation of the manuscript.

Participating investigators will be recognised in publication as contributors under the banner of the ***P. vivax*****Fever Study Group** unless they are a member of the Writing Committee, in which case they will be recognised in publication as named authors according to the ICMJE guidelines. The results of this study will be published in a peer-reviewed international journal and presented at international meetings with a focus on tropical medicine or malaria to inform other researchers and policy-makers.

**9. Potential Policy Outcome**

The data provided by this analysis will contribute to knowledge of *P. vivax* pyrogenic thresholds and will inform the development and evaluation of diagnostic tests.

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**11. Annex**

List of available co-variates.

|  |  |  |
| --- | --- | --- |
| **Category of Variable** | **Variable** | **Essential / Desirable** |
| **General** | Date of enrolment | Desirable |
|  | Follow-up duration | Desirable |
|  | Active and/or passive case detection during follow-up | Desirable |
|  | Recurrence rate  | Desirable |
|  | Date of recurrence(s) | Desirable |
| **Location** | **Study location** | **Essential** |
|  | GPS co-ordinates of location | Desirable |
|  | Study site details | Desirable |
| **Demographics** | **Age** | **Essential** |
|  | **Sex** | **Essential** |
|  | Pregnancy Status | Desirable |
| **Clinical history** | **Acute history of fever (within the last 72 hours)** **per parasitaemic episode\*** | **Essential** |
|  | Malaria history in the last year | Desirable |
| **Examination** | **Temperature per parasitaemic episode\*** | **Essential** |
|  | Weight | Desirable |
|  | Height | Desirable |
|  | Mid-Upper Arm Circumference (MUAC) | Desirable |
| **Microscopy** | **Method of calculation or units of parasitaemia** | **Essential** |
|  | **Asexual parasitaemia per parasitaemic episode** | **Essential** |
|  | **Species of parasitaemia per parasitaemic episode** | **Essential** |
|  | Microscopy quality assurance information | Desirable |
|  | Number of recurrences per participant | Desirable |
| **Other laboratory tests** | Haemoglobin at enrolment and recurrence | Desirable |
| **Treatment** | Schizontocidal treatment(s) tested | Desirable |
|  | Administration of schizontocidal treatment for asymptomatic recurrences | Desirable |
|  | 8-aminoquinoline treatment administered | Desirable |
|  | Timing of 8-aminoquinoline treatment | Desirable |

**\* Ideally both of these variables, although one of the two would be sufficient.**