Study Protocol
Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut 89, 1330 Rixensart,
Belgium

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
Date of protocol	Final Version 1: 21 January 2014
Date of protocol amendment/*administrative change*	Amendment 1 Final: 21 September 2016 Amendment 2 Final: 14 September 2018 **Administrative Change 1 Final: 28 October 2021**
EU PAS Register No.	***EUPAS43920***
Product reference	***H0002300***
Procedure number	***Not allocated***
Joint PASS	***No***
Title	Epidemiology study of malaria transmission intensity in sub-Saharan Africa.
Detailed Title	An epidemiology study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in catchment areas of two interventional studies pre- and post RTS,S/AS01$_E$ introduction (EPI-MAL-002 and EPI-MAL-003) to assess, in field conditions, vaccine benefit:risk in children in sub-Saharan Africa.
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- PPD Oversight Data Manager
- PPD Study Data Manager
- PPD Study Statistician

GSK Biologicals' protocol template for observational studies and interventional studies without administration of medicinal products as described in a research protocol based on the Protocol Document Standard version 14.1.2

(Administrative Change 1, 28 October 2021)

Protocol Administrative Change 1 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
Date of protocol administrative change	Administrative Change 1 Final: 28 October 2021
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Sponsor signatory	François Roman Clinical & Epidemiology Project Lead, DDW Vaccines

Signature _____

Date _____

Protocol Administrative Change 1 Rationale

Administrative Change number: Administrative Change 1
Rationale/background for changes: EPI-MALARIA-005 is referenced as a category 3 study in the Risk Management Plan (RMP) and was initially not classified as a PASS. In order to align with the GVP Module V Revision 2, where all category 3 studies assessing a risk are now classified as PASS, this study has been reclassified as a PASS. This administrative change to the protocol has been put in place to add additional information to comply with the ENCePP Checklist for study protocols, comprising adding 'EU PAS Register No.', 'Product reference', 'Procedure number' and Joint PASS status on the title page, and dates for study milestones to 'Study Design Overview'. Additionally, according to EU regulation, the definition of end of study (EoS) must be included in the clinical protocol, and the study report submitted in a predefined timeframe based on the EoS milestone. Per GSK policies, a summary of the study results have to be publicly disclosed, the reference milestone for which is based on the primary completion date (PCD). The definitions for the EoS and PCD have been clarified.

Protocol Administrative Change 1 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, with the terms of the study agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at the site(s).
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) or other applicable guidelines and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor, and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
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Investigator name	_____
Signature	_____
Date	_____

Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals

Rue de l'Institut 89, 1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Study Contact for Reporting of a Serious Adverse Event (SAE)

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to protocol Section 6.3.2.

SYNOPSIS

Detailed Title	An epidemiology study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in catchment areas of two interventional studies pre- and post RTS,S/AS01$_E$ introduction (EPI-MAL-002 and EPI-MAL-003) to assess, in field conditions, vaccine benefit:risk in children in sub-Saharan Africa.
Rationale for the study	Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of meningitis, protocol defined adverse events of special interest (AESI), and of other adverse events leading to hospitalisation or death, in children. The first study, EPI-MAL-002, is a baseline surveillance study prior to RTS,S/AS01$_E$ authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/AS01$_E$ safety, as well as vaccine effectiveness and impact, and will only start when RTS,S/AS01$_E$ is authorised and implemented in the country. The World Health Organi*s*ation's (WHO's) Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC) recommended pilot implementations of RTS,S/AS01$_E$ in children of 5–17 months of age, in parts of 3-5 sub-Saharan African countries, administering 3 doses of the vaccine to children aged 5-9 months of age in areas of moderate-to-high transmission of malaria with a fourth dose 15-18 months later. The first vaccine introduction is foreseen in 2018. The secondary endpoints of these studies also include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. Health and Demographic Surveillance System (HDSS) (or equivalent system) sites[1] in countries in sub-Saharan Africa will participate in these studies, enrolling approximately 30 000 children <5 years of age in EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003. This epidemiology study (EPI-MAL-005) is planned to run in parallel with these two studies, enrolling from the same HDSS ***(***or equivalent system) populations. The primary objectives of this study are to produce longitudinal estimates of parasite prevalence in humans, and record malaria control measure

[1] Throughout the document, the terms of site, cluster and centre are used interchangeably, having the same meaning.

usage in areas where EPI-MAL-002 and EPI-MAL-003 studies will take place. As requested by WHO/JTEG, parasite prevalence as an indicator of malaria transmission intensity (MTI) may also contribute to the evidence base for vaccine recommendation in different MTI settings. It is expected that RTS,S/AS01$_E$ vaccine introduction through national immunisation systems will lead to a reduction in the incidence of malaria disease in vaccinated subjects in EPI-MAL-003 when compared to baseline rates recorded in EPI-MAL-002. Annual fluctuations in malaria incidence occur as a result of changes in transmission intensity, which may be caused by changes in environmental factors such as rainfall or changes in usage of other malaria control interventions. Therefore, by taking into account these variations in MTI and malaria control intervention coverage, it will be possible to estimate more accurately the vaccine impact on clinical disease during EPI-MAL-003. These data will also allow for an assessment of any association between vaccination and gametocyte carriage in the 0-2 year age group, as an indicator of the potential effect of the vaccine on malaria transmission.

Outside of the controlled setting of a clinical trial, there is the risk that usage of malaria control interventions such as indoor residual spraying and bednets may change following vaccine introduction. Vaccine introduction may lead to the misperception that other interventions are no longer required or may alter health seeking behaviours for fever, and thus are potential confounders of the effect of the vaccine on clinic-identified malaria and therefore need to be monitored.

Although not yet considered as the gold standard, NAAT-based (nucleic acid amplification test) techniques can detect infections of lower malaria parasite density and quantification of parasitaemia than is achievable by microscopy. Identification of gametocytes by microscopy is inherently challenging, thus their presence is often missed, even by highly trained technicians. Therefore, additional analysis of collected blood samples by NAAT will allow for the detection of lower density parasite infections and will be a more sensitive measure of changes in both parasite and gametocyte prevalence and density, thereby providing greater insight into potential changes upon vaccine implementation. This will also provide comparability of data collected in this study with the technique likely to be favoured in clinical trials in the future.

These data will enable a more complete assessment of the benefits and risks of vaccine introduction, and thereby more insight into the potential vaccine impact in

EPI-MAL-002/-003, by adjusting incidence data for overall changes in transmission and other malaria control intervention coverage, and assist generalisation of results to other populations.

Objectives

Co-Primary

In subjects aged 6 months to <10 years:

- To obtain longitudinal estimates of *P. falciparum* parasite prevalence in order to characterise malaria transmission intensity in a standardised way at centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa.
- To obtain longitudinal estimates of the use of malaria control interventions in centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa.

Secondary

In subjects aged 6 months to <10 years:

- To estimate trends in longitudinal estimates of the parasite prevalence of *P. falciparum* by vaccine eligible or ineligible subgroups and overall.
- To obtain longitudinal estimates of the prevalence of *Plasmodium* species other than *P. falciparum*; overall and by age group.
- To estimate longitudinal trends in receipt and timing of the third dose of DTP/HepB/Hib and the first dose of measles EPI vaccines, at around 14 weeks and 9 months of age respectively, as appropriate by country.
- To describe changes in care seeking behaviours for reported fever or malaria in the previous 14 days.
- To assess within-site geographical heterogeneity in malaria transmission intensity.
- To describe individual malaria prevention measures and risk factors for clinical malaria according to the parasite density observed.

Tertiary

In subjects aged 6 months to <10 years:

- To compare asexual and sexual (gametocyte) parasitaemia (qualitative and quantitative-density) in the RTS,S/AS01$_E$ vaccinated and unvaccinated subjects.
- To compare asexual and sexual (gametocyte) parasitaemia (qualitative and semi-quantitative-density) when measured by microscopy or NAAT.

Study design (Administrative Change 1, 28 October 2021)

- Type of design: A multi-centric, epidemiology longitudinal cross-sectional study at centres in sub-Saharan Africa that are participating in GSK's EPI-MAL-002 and EPI-MAL-003 studies.
- There will be no study vaccine administered in this epidemiology study.
- Study population: Subjects 6 months to <10 years of age.
- Type of study: self-contained
- All medications that may influence malaria parasitaemia within 14 days prior to each survey will be recorded.
- Axillary body temperature of all subjects at the time of the survey will be recorded.
- Biological samples: A capillary blood sample will be obtained for evaluation of malaria infection by blood slide and NAAT. In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in the last 24 hours or other symptoms/signs of clinical malaria, a rapid diagnostic test (RDT) will be conducted. If the RDT is positive, treatment will be given according to National guidelines. If a subject for whom no RDT was required is identified as being parasite positive following microscopy, National guidelines should be followed for clinical management of the subject.
- Microscopy and NAAT will be used to evaluate the level of asexual and sexual parasitaemia.
- Serious adverse events (SAEs) associated with the study procedure (capillary blood sampling) will be collected.
- Using Geographic Information System (GIS) to determine geographical variability in MTI locally: Study areas will be mapped by villages using grid referencing. Subjects will be attributed to their village, however, to avoid PII (personally identifiable information); small

villages will be grouped when the number of participants is less than 10, so that it is not possible to identify one subject from one village. Therefore the study area will be divided into segments with a minimum of 10 subjects by segment regrouping villages by proximity if needed. Villages will be grouped at the time of random sampling of the study population. A map will be drawn showing the location of numbered segments, each numbered with a unique ID code and containing one or more villages. This map will NOT be submitted to GSK, but segments will be reported by their ID code and surface (km^2) and subjects will be attributed to a segment code in the study database.

- Each study site will be requested annually to provide centre specific information about interventions from the malaria control programme in the study area to provide meteorological data for the study site such as rainfall and temperature. The information will be collected in the form of a questionnaire that will be recorded in a separate database to that for subject-specific data.
- Data collection: Electronic Case Report Form (eCRF).
- This study will involve up to 10 annual cross sectional surveys during malaria peak transmission with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies.
- Duration of the study: up to 10 years with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies:
  - Epoch 001: Survey 1 at Year 1
  - Epoch 002: Survey 2 at Year 2
  - Epoch 003: Survey 3 at Year 3
  - Epoch 004: Survey 4 at Year 4
  - Epoch 005: Survey 5 at Year 5.
  - Epoch 006: Survey 6 at Year 6.
  - Epoch 007: Survey 7 at Year 7.
  - Epoch 008: Survey 8 at Year 8.
  - Epoch 009: Survey 9 at Year 9
  - Epoch 010: Survey 10 at Year 10
- ***Primary completion date (PCD): the date of final collection of data for all primary outcomes/endpoints.***

- ***End of Study (EoS): the date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/Max)	Epochs									
		Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 4	Epoch 005 Survey 5	Epoch 006 Survey 6	Epoch 007 Survey 7	Epoch 008 Survey 8	Epoch 009 Survey 9	Epoch 010 Survey 10
Study site	6 months / 9 years	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects

***Milestones* (Administrative Change 1, 28 October 2021)**

Milestone	*Planned/Actual dates*
Start of data collection	*Q4 2014**
Last subject last visit (LSLV)	*Q3 2024*
End of data collection	*Q1 2025*
Interim report 1 (Surveys 1 & 2)	*Q3 2017**
Interim report 2 (Surveys 3, 4 & 5)	*Q1 2021**
Registration in the EU PAS register	*Q4 2021**
Final report of study results	*Q4 2025*

****Actual dates***

Discussion of study design

Several methods of estimating MTI exist, including entomological inoculation rates (EIR), serological conversion rates (SCR) and blood parasite prevalence. The methodology and interpretation of SCR to classify the intensity of malaria is not commonly used yet, and although the EIR is a standard method, the measure is challenging and interpretation and comparability of setting may be difficult due to vector heterogeneity. Parasite prevalence, although requiring trained staff for slide reading, provides a standardised and relatively easy to implement method to assess MTI in study sites of varied transmission intensity and is therefore the method of choice in this study.

At least 10 annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. Malaria transmission is highly seasonal so surveys will be carried out during the course of the rainy season preferably when rains decrease, the period of highest malaria transmission. Annual fluctuations may be seen in all endemic areas and a longitudinal assessment will allow for a better appreciation of the scale and trend of this annual variation. Endemicity is dependent on several environmental factors

including climate (rain and temperature), access to malaria care, and use of other malaria control interventions. Therefore, concurrent collection of data on these factors will be important for the interpretation of these study results and evaluation of vaccine impact.

This study will be conducted in parallel to EPI-MAL-002 and EPI-MAL-003 in order to assess parasite prevalence and malaria control measures before and after vaccine introduction.

The age group for enrolment (6 months to <10 years) has been selected in this study as this permits analysis of parasite prevalence according to the WHO definition (2-9 years) and by the JTEG requested age (<5 years).

Number of subjects

Total expected sample size: Per site and by survey is a maximum of 400 subjects aged 6 months to <5 years and a maximum of 200 subjects aged 5 to <10 years.

Endpoints

Primary

- Occurrence of *P. falciparum* parasitaemia (using microscopy)

  Criteria/definitions: infection with *P. falciparum* determined using a blood smear slide and determined using microscopy.

- Occurrence of malaria control interventions

  Criteria/definitions: malaria control interventions are mosquito net usage (including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN]), indoor residual spraying (IRS), seasonal malaria chemoprevention (SMC), intermittent preventative treatment in infants (IPTi), and ACT therapy received within the last 14 days.

Secondary

- Demography and history characteristics

  Criteria/definitions: gender, age, medical history.

- Occurrence of *Plasmodium* species other than *P. falciparum* (using microscopy)

  Criteria/definitions: infection with *Plasmodium* species other than *P. falciparum* determined using a blood smear slide and microscopy.

- Occurrence of uptake and timing of the third dose of DTP/HepB/Hib and the first measles EPI vaccines

  Criteria/definitions: vaccination record of receipt of dose 3 of the DTP/HepB/Hib and the first dose of the measles EPI vaccines.

- Occurrence of anti-malarial therapy

  Criteria/definitions: any anti-malarial therapy received in the last 14 days.

- Occurrence of measured fever

  Criteria/definitions: any measured fever at time of visit (axillary temperature ≥37.5°C).

- Occurrence of reported fever

  Criteria/definitions: any reported fever occurring in the last 24 hours.

- Occurrence of care seeking behaviour

  Criteria/definitions: occurrence of visits to health providers following reported fever or malaria in the previous 14 days.

- Geo-referencing characteristics

  Criteria/definitions: positioning of the subject's residence will be attributed to a segment with a unique ID from the grid referencing study area map in which the subject resides, where necessary, grouping small geographically proximate villages so that each segment has at least 10 study subjects to avoid PII, and proceeding as far as geographically appropriate.

- Occurrence of individual malaria prevention measures and risk factors

  Criteria/definitions: malaria prevention measures are repellents and local herbs not specifically recommended by the national programme and risk factors are rural/urban area, construction material for the house, floor and roof, type of eaves (open/closed), use of electricity and water source (distance from and type).

Tertiary

- Occurrence of RTS,S/AS01$_E$ vaccine doses

  Criteria/definitions: vaccination record of each dose received of the RTS,S/AS01$_E$ vaccine..

- Occurrence of *P. falciparum* parasitaemia (using NAAT)

Criteria/definitions: detection of *P. falciparum* on dried blood spot and determined using NAAT.

- Occurrence of sexual *P. falciparum* parasitaemia (using microscopy)

  Criteria/definitions: detection of sexual *P. falciparum* using a blood smear slide and determined using microscopy.

- Occurrence of sexual *P. falciparum* (using NAAT/QT-NASBA)

  Criteria/definitions: detection of sexual *P. falciparum* on dried blood spot and determined using NAAT.

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LIST OF ABBREVIATIONS

ACT	Artemisinin-based combination therapy
AESI	Adverse events of special interest
AMC	Academic Medical Center
ATP	According to protocol
CI	Confidence interval
CSP	Circumsporozoite protein
DTP	Diphtheria, tetanus, pertussis
eCRF	electronic Case Report Form
EIR	Entomological inoculation rates
EoS	***End of Study***
EPI	Expanded program on immunization
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCP	Good Clinical Practice
GIS	Geographic Information System
GPP	Good Pharmacoepidemiology Practice
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HDSS	Health and Demographic surveillance system
HepB	Hepatitis B
Hib	Heamophilus influenza type b
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IPTi	Intermittent preventative treatment in infants

IPTp	Intermittent preventative treatment in pregnancy
IRB	Institutional Review Board
IRS	Indoor residual spraying
ITN	Insecticide-treated net
JTEG	Joint technical expert group
LAR	Legally acceptable representative
LL	Lower limit
LLIN	Long lasting insecticidal nets
LSLV	***Last Subject Last Visit***
MPAC	Malaria Policy Advisory Committee
MTI	Malaria transmission intensity
MVI	PATH-Malaria Vaccine Initiative
MVIP	Malaria Vaccine Implementation Programme
NAAT	Nucleic acid amplification test
NC/NT-SAE	Non-communicable and non-traumatic serious adverse events
P. falciparum	*Plasmodium falciparum*
PCD	***Primary Completion Date***
PII	Personally identifiable information
PRAC	Pharmacovigilance Risk Assessment Committee
QT-NASBA	Quantitative nucleic acid sequence-based amplification
QT-PCR	Quantitative polymerase chain reaction
RDT	Rapid diagnostic test
RR	Relative risk
RSE	Relative standard error
RT-PCR	Reverse transcriptase polymerase chain reaction

RTS	Hybrid protein comprising HBs (hepatitis B surface antibody) and CSP portions
RTS,S	Particulate antigen, containing both RTS and HBs proteins
SAE	Serious adverse event
SAGE	Strategic Advisory Group of Experts
SCR	Serological conversion rate
SDV	Source document verification
SMC	Seasonal malaria chemoprevention
SOP	Standard operating procedure
SPM	Study procedures manual
TFL	Tables, figures, listings
UL	Upper limit
WHO	World Health Organisation

(Administrative Change 1, 28 October 2021)

GLOSSARY OF TERMS

Anonymised data:	Information about an individual that GSK or a third party cannot reasonably attribute to the individual, or could only attribute to the individual by expending a disproportionate amount of time, effort or expense (e.g. de-identified or aggregated information). For the purpose of this policy, Key-Coded personally identifiable information shall not be considered Anonymised Information
Child in care:	A child who has been placed under the control or protection of an agency, organisation, institution or entity by the courts, the government or a government body, acting in accordance with powers conferred on them by law or regulation. The definition of a child in care can include a child cared for by foster parents or living in a care home or institution, provided that the arrangement falls within the definition above. The definition of a child in care does not include a child who is adopted or has an appointed legal guardian.
Catchment area:	In this study, the catchment area is defined as the area of the HDSS (or equivalent system) study site participating in the EPI-MAL-002 and EPI-MAL-003 studies.
Care seeking behaviour:	When and where and at what time a child is taken for treatment for reported fever or malaria in the last 14 days.
DTP/HepB/Hib:	This refers to DTP, or DTP-Hep B (tetravalent) or DTP-HepB-Hib (pentavalent).
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of study (EoS):	***For studies with collection of Human Biologicals Samples, EoS is defined as:*** ***The date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.
Epoch:	An epoch is a self-contained set of consecutive time points or a single time point from a single protocol. Self-

	contained means that data collected for all subjects at all time points within that epoch allows to draw a complete conclusion. Typical examples of epochs are retrospective data collection and prospective data collection, etc.
eTrack:	GSK Biologicals' tracking tool for clinical/ epidemiological trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Section 7.3 for details on criteria for evaluability).
First dose measles:	First measles containing vaccine.
Malaria control interventions:	Any intervention or control measure targeted at reducing malaria transmission or preventing malaria disease [World Health Organisation, 2013]. This includes ITN, LLIN, IRS, SMC, IPTi, IPTp and ACTs.
Malaria prevention measures:	Any individual intervention used to prevent malaria and not specifically recommended by the malaria program. This includes mosquito coils and local herbs.
Primary completion date (PCD):	***PCD is defined as the date of final collection of data for all primary outcomes/endpoints.***
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study. NB Any change that falls under the definition of a protocol amendment (e.g., a change that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study) MUST be prepared as an amendment to the protocol.
Self-contained study:	Study with objectives not linked to the data of another study.

Site Monitor:	An individual assigned by the sponsor who is responsible for assuring the proper conduct of epidemiological studies at one or more investigational sites.
Study population:	Sample of population of interest.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the epidemiological study or a person about whom some medical information has been recorded in a database.
Subjects at least 6 months of age:	Six months old is defined as the same day as the date of birth occurring 6 calendar months later (e.g. if born on the 8th January, a child becomes 6 months old on the 8th July).
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
Surveillance:	The ongoing systematic collection, collation, analysis, and interpretation of descriptive epidemiological health data on a specific disease. Surveillance can monitor incidence and/or prevalence, and/or inform about when and where health problems are occurring and who is affected.
Vaccine eligible age:	Vaccine eligible is defined as those subjects that on the basis of age would be eligible for RTS,S/AS01$_E$ vaccination, even if vaccine is unavailable at the time of assessment. The age will depend on the label for RTS,S/AS01$_E$ vaccination in the country. (At the time of study start, there will be no available vaccination with RTS,S/AS01$_E$).
Vaccine ineligible age:	Vaccine ineligible is defined as those subjects that on the basis of age would be ineligible for RTS,S/AS01$_E$ vaccination, regardless of vaccine availability at the time of assessment. The age will depend on the label for RTS,S/AS01$_E$ vaccination in the country.

(Administrative Change 1, 28 October 2021)

1. INTRODUCTION

1.1. Background

GlaxoSmithKline (GSK) Biologicals in partnership with the PATH-Malaria Vaccine Initiative (MVI) is developing a *Plasmodium falciparum* malaria vaccine for routine immunisation of infants and children living in malaria-endemic areas.

GSK Biologicals has formulated the candidate pre-erythrocytic *P. falciparum* malaria vaccine, RTS,S/AS01$_E$, which is currently under evaluation. The vaccine consists of sequences of the circumsporozoite (CS) protein and hepatitis B surface antigen (HBsAg) adjuvanted with AS01 (liposome formulation with MPL and QS21 immunostimulants).

In the pivotal Phase III study (Malaria-055), efficacy of the candidate malaria vaccine RTS,S/AS01$_E$ against first or only episodes of clinical malaria over a follow-up of 12 months in children aged 5-17 months was 55.8% ($p<0.0001$), and in coadministration with DTPwHepB/Hib vaccine at 6, 10 and 14 weeks of age was 31.3% ($p<0.0001$). Phase II and early Phase III data suggest it has an acceptable safety profile when given to young children and infants in co-administration with EPI routine vaccines. Please refer to the current Investigator Brochure for a review of the pre-clinical and clinical studies, and the potential risks and benefits of RTS,S/AS02 and RTS,S/AS01.

1.2. Rationale for the study

Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of meningitis, protocol defined adverse events of special interest (AESI), and of other adverse events leading to hospitalisation or death, in children. The first study, EPI-MAL-002, is a baseline surveillance study prior to RTS,S/AS01$_E$ authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/AS01$_E$ safety, as well as vaccine effectiveness and impact, and will only start when RTS,S/AS01$_E$ is authorised and implemented in the country. The World Health Organisation's (WHO's) Strategic Advisory Group of Experts (SAGE) on immunization and the Malaria Policy Advisory Committee (MPAC) recommended pilot implementations of RTS,S/AS01$_E$ in children of 5–17 months of age, in parts of 3-5 sub-Saharan African countries, administering 3 doses of the vaccine to children aged 5-9 months of age in areas of moderate-to-high transmission of malaria with a fourth dose 15-18 months later. The first vaccine introduction is foreseen in 2018. The secondary endpoints of these studies also include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. Health and Demographic Surveillance System (HDSS) (or equivalent system) sites[2] in countries in ***s***ub-Saharan Africa will participate in these studies,

[2] Throughout the document, the terms of site, cluster and centre are used interchangeably, having the same meaning.

enrolling approximately 30 000 children <5 years of age in EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003.

This epidemiology study (EPI-MAL-005) is planned to run in parallel with these two studies, enrolling from the same HDSS (or equivalent system) populations. The primary objectives of this study are to produce longitudinal estimates of parasite prevalence in humans, and record malaria control measure usage in areas where EPI-MAL-002 and EPI-MAL-003 studies will take place. As requested by WHO/JTEG, parasite prevalence as an indicator of malaria transmission intensity (MTI) may also contribute to the evidence base for vaccine recommendation in different MTI settings. It is expected that RTS,S/AS01$_E$ vaccine introduction through national immunisation systems will lead to a reduction in the incidence of malaria disease in vaccinated subjects in EPI-MAL-003 when compared to baseline rates recorded in EPI-MAL-002. Annual fluctuations in malaria incidence occur as a result of changes in transmission intensity, which may be caused by changes in environmental factors such as rainfall or changes in usage of other malaria control interventions. Therefore, by taking into account these variations in MTI and malaria control intervention coverage, it will be possible to estimate more accurately the vaccine impact on clinical disease during EPI-MAL-003. These data will also allow for an assessment of any association between vaccination and gametocyte carriage in the 0-2 year age group, as an indicator of the potential effect of the vaccine on malaria transmission.

Outside of the controlled setting of a clinical trial, there is the risk that usage of malaria control interventions such as indoor residual spraying and bednets may change following vaccine introduction. Vaccine introduction may lead to the misperception that other interventions are no longer required or may alter health seeking behaviours for fever, and thus are potential confounders of the effect of the vaccine on clinic-identified malaria and therefore need to be monitored.

Although not yet considered as the gold standard, NAAT-based (nucleic acid amplification test) techniques can detect infections of lower malaria parasite density and give more accurate quantification of parasitaemia than is achievable by microscopy. Identification of gametocytes by microscopy is inherently challenging, thus their presence is often missed, even by highly trained technicians. Therefore, additional analysis of collected blood samples by NAAT will allow for the detection of lower density parasite infections and will be a more sensitive measure of changes in both parasite and gametocyte prevalence and density, thereby providing greater insight into potential changes upon vaccine implementation. This will also provide comparability of data collected in this study with the technique likely to be favoured in clinical trials in the future.

These data will enable a more complete assessment of the benefits and risks of vaccine introduction, and thereby more insight into the potential vaccine impact in EPI-MAL-002/-003, by adjusting incidence data for overall changes in transmission and other malaria control intervention coverage, and assist generalisation of results to other populations.

2. OBJECTIVES

2.1. Co-Primary objectives

In subjects aged 6 months to <10 years:

- To obtain longitudinal estimates of *P. falciparum* parasite prevalence in order to characterise malaria transmission intensity in a standardised way at centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa.
- To obtain longitudinal estimates of the use of malaria control interventions in centres conducting the EPI-MAL-002 and EPI-MAL-003 studies before and after the introduction of the malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa.

Refer to Section 7.1.1 for the definition of the primary endpoints.

2.2. Secondary objectives

In subjects aged 6 months to <10 years:

- To estimate trends in longitudinal estimates of the parasite prevalence of *P. falciparum* by vaccine eligible or ineligible subgroups and overall.
- To obtain longitudinal estimates of the prevalence of *Plasmodium* species other than *P. falciparum*; overall and by age group.
- To estimate longitudinal trends in receipt and timing of the third dose of DTP/HepB/Hib and the first dose of measles EPI vaccines, at around 14 weeks and 9 months of age respectively, as appropriate by country.
- To describe changes in care seeking behaviours for reported fever or malaria in the previous 14 days.
- To assess within-site geographical heterogeneity in malaria transmission intensity.
- To describe individual malaria prevention measures and risk factors for clinical malaria according to the parasite density observed.

Refer to Section 7.1.2 for the definition of the secondary endpoints.

2.3. Tertiary objectives

In subjects aged 6 months to <10 years:

- To compare asexual and sexual (gametocyte) parasitaemia (qualitative and quantitative-density) in the RTS,S/AS01$_E$ vaccinated and unvaccinated subjects.
- To compare asexual and sexual (gametocyte) parasitaemia (qualitative and semi-quantitative-density) when measured by microscopy or NAAT.

Refer to Section 7.1.3 for the definition of the tertiary endpoints.

3. STUDY DESIGN OVERVIEW

Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 5.4), are essential and required for study conduct.

- Type of design: A multi-centric, epidemiology longitudinal cross-sectional study at centres in ***s***ub-Saharan Africa that are participating in GSK's EPI-MAL-002 and EPI-MAL-003 studies.
- There will be no study vaccine administered in this epidemiology study.
- Study population: Subjects 6 months to <10 years of age.
- Type of study: self-contained
- All medications that may influence malaria parasitaemia within 14 days prior to each survey will be recorded.
- Axillary body temperature of all subjects at the time of the survey will be recorded.
- Biological Samples: A capillary blood sample will be obtained for evaluation of malaria infection by blood slide and NAAT. In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in the last 24 hours or other symptoms/signs of clinical malaria, a rapid diagnostic test (RDT) will be conducted. If the RDT is positive, treatment will be given according to National guidelines. If a subject for whom no RDT was required is identified as being parasite positive following microscopy, National guidelines should be followed for clinical management of the subject.
- Microscopy and NAAT will be used to evaluate the level of asexual and sexual parasitaemia.
- Serious adverse events (SAEs) associated with the study procedure (capillary blood sampling) will be collected.
- Using Geographic Information System (GIS) to determine geographical variability in MTI locally: Study areas will be mapped by villages using grid referencing. Subjects will be attributed to their village, however, to avoid PII (personally identifiable information), small villages will be grouped when the number of participants is less than 10, so that it is not possible to identify one subject from one village. Therefore the study area will be divided into segments with a minimum of 10 subjects by segment regrouping villages by proximity if needed. Villages will be grouped at the time of random sampling of the study population. A map will be drawn showing the location of numbered segments, each numbered with a unique ID code and containing one or more villages. This map will NOT be submitted to GSK, but segments will be reported by their ID code and surface (km^2) and subjects will be attributed to a segment code in the study database.
- Each study site will be requested annually to provide centre specific information about interventions from the malaria control program in the study area and, if facilities are available, to provide meteorological data for the study site such as

rainfall and temperature. The information will be collected in the form of a questionnaire that will be recorded in a separate database to that for subject-specific data.

- Data collection: Electronic Case Report Form (eCRF).
- This study will involve up to 10 annual cross sectional surveys during malaria peak transmission with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies.
- Duration of the study: up to 10 years with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies:
  - Epoch 001: Survey 1 at Year 1
  - Epoch 002: Survey 2 at Year 2
  - Epoch 003: Survey 3 at Year 3
  - Epoch 004: Survey 4 at Year 4
  - Epoch 005: Survey 5 at Year 5.
  - Epoch 006: Survey 6 at Year 6.
  - Epoch 007: Survey 7 at Year 7.
  - Epoch 008: Survey 8 at Year 8.
  - Epoch 009: Survey 9 at Year 9
  - Epoch 010: Survey 10 at Year 10
- ***Primary completion date (PCD): the date of final collection of data for all primary outcomes/endpoints.***
  Refer to GLOSSARY OF TERMS for the definition of primary completion date.
- ***End of Study (EoS): the date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***
  Refer to GLOSSARY OF TERMS for the definition of EoS.

Table 1 Study groups and epochs foreseen in the study (Amended 14 September 2018)

Study Group	Age (Min/Max)	Epochs									
		Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 4	Epoch 005 Survey 5	Epoch 006 Survey 6	Epoch 007 Survey 7	Epoch 008 Survey 8	Epoch 009 Survey 9	Epoch 010 Survey 10
Study site	6 months / 9 years	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects

- ***Milestones***

Milestone	*Planned/Actual dates*
Start of data collection	*Q4 2014**
Last subject last visit (LSLV)	*Q3 2024*
End of data collection	*Q1 2025*
Interim report 1 (Surveys 1 & 2)	*Q3 2017**
Interim report 2 (Surveys 3, 4 & 5)	*Q1 2021**
Registration in the EU PAS register	*Q4 2021**
Final report of study results	*Q4 2025*

Actual dates

(Administrative Change 1, 28 October 2021)

3.1. Discussion of study design

Several methods of estimating MTI exist, including entomological inoculation rates (EIR), serological conversion rates (SCR) and blood parasite prevalence. The methodology and interpretation of SCR to classify the intensity of malaria is not commonly used yet, and although the EIR is a standard method, the measure is challenging and interpretation and comparability of setting may be difficult due to vector heterogeneity. Parasite prevalence, although requiring trained staff for slide reading, provides a standardised and relatively easy to implement method to assess MTI in study sites of varied transmission intensity and is therefore the method of choice in this study.

At least 10 annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. Malaria transmission is highly seasonal so surveys will be carried out during the course of the rainy season preferably when rains decrease, the period of highest malaria transmission. Annual fluctuations may be seen in all endemic areas and a longitudinal assessment will allow for a better appreciation of the scale and trend of this annual variation. Endemicity is dependent on several environmental factors including climate (rain and temperature), access to malaria care, and use of other malaria control interventions. Therefore, concurrent collection of data on these factors will be important for the interpretation of these study results and evaluation of vaccine impact.

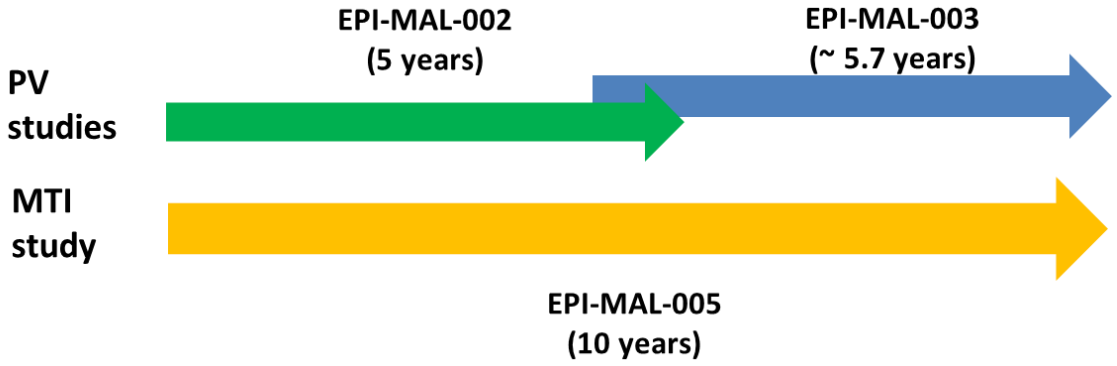
This study will be conducted in parallel to EPI-MAL-002 and EPI-MAL-003 in order to assess parasite prevalence and malaria control measures before and after vaccine introduction.

The age group for enrolment (6 months to <10 years) has been selected in this study as this permits analysis of parasite prevalence according to the WHO definition [WHO, 1963] (2-9 years) and by the JTEG requested age (<5 years).

4. STUDY POPULATION

4.1. Number of subjects / centres

Subjects 6 months to <10 years of age living in HDSS (or equivalent system) catchment areas of the sites participating in the EPI-MAL-002 and EPI-MAL-003 studies are eligible for enrolment (see Section 4.1.1). Subjects already enrolled in EPI-MAL-002 and EPI-MAL-003 are eligible for participation in this study.



Each study site will enroll approximately 600 subjects per survey. These subjects will be selected at random from the HDSS (or equivalent system) population listings prepared at each site. The selection process will be repeated every year meaning that the subjects will be different in each cross-sectional survey except if they are re-selected in a subsequent survey by chance. The population listings generated from the demographic surveillance will allow for sampling of the required subjects according to stratification by age group as follows (all subject numbers are approximately plus or minus 5 children):

- 60 children aged 6 months to <1 year
- 120 children aged 1 year
- 120 children aged 2 years
- 50 children aged 3 years
- 50 children aged 4 years
- 40 children aged 5 years
- 40 children aged 6 years
- 40 children aged 7 years
- 40 children aged 8 years
- 40 children aged 9 years

Therefore, the total expected sample size per site and by survey is a maximum of 400 subjects aged 6 months to <5 years and a maximum of 200 subjects aged 5 to <10 years. Refer to Section 7.2 for a detailed description of the criteria used in the estimation of sample size.

4.1.1. Recruitment of study centres / investigators

The ancillary EPI-MAL-005 study will be conducted in sites defined in the EPI-MAL-002 and EPI-MAL-003 protocols. At the time of original protocol finalisation, identified study sites were located in Burkina Faso, Ghana, Kenya, Senegal, and Tanzania.

Following the WHO's SAGE on immunization and the MPAC recommendations of pilot implementations of RTS,S/AS01$_E$ in 3-5 distinct settings in sub-Saharan Africa restricted to moderate-to-high transmission of malaria, site selection in some of the initially chosen EPI-MAL-002, EPI-MAL-003 and EPI-MAL-005 study sites located in low endemicity settings (i.e. in Senegal and Tanzania) had to be terminated. In April 2017, the WHO Regional Office for Africa announced that the RTS,S/AS01$_E$ vaccine will be first introduced in 3 countries (Ghana, Kenya and Malawi) through the Malaria Vaccine Implementation Programme (MVIP). Selection of the clusters that are/will participate in GSK's baseline, Phase IV and ancillary studies (i.e. EPI-MAL-002, EPI-MAL-003 and EPI-MAL-005, respectively), being fully embedded in the MVIP, depends on the cluster identification process led by the Ministries of Health according to WHO guidance. They have been, or will be, selected as follows:

- Sites have been, or will be, selected from the 3 countries where the RTS,S/AS01$_E$ vaccine will be implemented (Ghana, Kenya and Malawi). Burkina Faso sites that started EPI-MAL-005 will early terminate the conduct of the study following Survey 4; data from these sites will be presented in the interim report planned following Survey 5.
- As currently planned in the MVIP and according to WHO guidance, 4 study sites (corresponding to 4 clusters of the MVIP) in each of the 3 countries selected for the RTS,S/AS01$_E$ pilot implementation programme (12 study sites/clusters in total) are planned to be part of EPI-MAL-005.
- Of note, all study sites are submitted to a comprehensive scientific and operational study site assessment conducted by GSK, which will determine study feasibility in those sites.

In summary, selection of sites will be performed in EPI-MAL-002 and EPI-MAL-003 studies following Ministries of Health pre-selection and according to WHO guidance, to include 4 sites in each of the 3 countries selected for the MVIP. EPI-MAL-005 is/will be conducted in study sites conducting EPI-MAL-002 and/or EPI-MAL-003.

4.1.2. Sampling methods

All participating centres will have a HDSS (or equivalent system) in place. In general, any variation in sampling procedure should follow as closely as possible the Guidelines

of the [Roll Back Malaria Monitoring and Evaluation Reference Group, 2005]. The following steps serve as an example of a sampling method.

1. Generate a list from the HDSS (or equivalent system) database of all children aged 6 months to <10 years.
2. Order the list of subjects by age, then by village within each age category.
3. Divide the total number of children for each age category by the sample needed in that age category, including 10% or more, according to local variations, extra to reflect non-response/refusal. This gives the size of the sequence for that age category (e.g. every xth subject, where x=total number of children for age category/number to be selected in the age category).
4. If the sequence (x) is 20 for example, select randomly a first subject among the 20 first subjects.
5. From this subject, pick every 20th subject until the end of that age category.
6. Repeat steps 3-5 for each age category.

If a subject is not found during the study team visit (e.g. possible migration, hospitalisation, …) three attempts should be made to reach the subject at home to minimise sampling bias, before the subject's enrolment is abandoned. It is likely there will be some non-response as a result of migration or refusal by the parents/LAR and this has been taken into account in the sample size calculation.

The household of each randomly selected subject should be visited by a study team that will be able to carry out the protocol procedures in field conditions e.g. informed consent form (ICF) procedure, blood sample collection, questionnaire administration and data recording. In order to enable logistically feasible, but methodologically rigorous, sampling the field team should work their way through the sampling at their own discretion in a way that minimises travel to and between sampling points, e.g. in order of proximity of subjects or geographical sequence.

Each study site will enrol approximately 600 subjects per survey. To allow for 10% non-response 660 subjects should therefore be randomly selected from the HDSS (or equivalent system) database distributed over all age categories as described in Section 4.1. In order to meet enrolment timelines, the sampling scheme could be implemented in the following fashion: 3 survey teams, each comprising two individuals, can be in the field 5 days per week for a period of 8 weeks. Each day in the field, a minimum of 5 subjects should be visited per team. Therefore, over the course of the 8 weeks, a minimum of 600 visits can be made. Though more time may be required if large numbers of subjects require repeat visits, teams may achieve more visits per day than proposed here if subjects are in close proximity or in the same household.

4.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardise the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects' whose parent(s)/Legally Acceptable Representative(s) [LAR(s)], in the opinion of the investigator, can and will comply with the requirements of the protocol.
- A male or female 6 months to <10 years of age at the time of survey.
- Signed informed consent or thumbprinted and witnessed informed consent obtained from the parent(s)/LAR(s) of the child.

4.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardise the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Child in care

  Please refer to the glossary of terms for the definition of child in care.
- Current active participation in any trial involving administration of an investigational malaria vaccine or malaria drug.

5. CONDUCT OF THE STUDY

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), Guidelines for Good Pharmacoepidemiology Practices (GPP) [ISPE, 2015], other applicable guidelines, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

The study has been designed and will be conducted in accordance with the ICH Harmonised Tripartite Guideline for clinical investigation of medicinal products in the paediatric population (ICH E11) and all other applicable ethical guidelines.

GSK will obtain favourable opinion/approval to conduct the study prior to a site initiating the study in that country or will document that neither a favourable opinion nor an approval to conduct the study is needed.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject's parent(s)/LAR(s) informed consent and subject informed assent, as appropriate.
- Investigator reporting requirements as stated in the protocol.

GSK Biologicals will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written or thumbprinted informed consent must be obtained from each subject's parent(s)/LAR(s) or the impartial witness and subject informed assent, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the applicable ICH GCP or other applicable guidelines, and GSK Biologicals required elements. While it is strongly recommended that this model ICF be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

5.2. Subject identification

Subject numbers will be assigned sequentially to subjects consenting to participate/to be included in the study, according to the range of subject numbers allocated to each study centre.

5.3. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying Study Procedures Manual (SPM). The SPM provides the investigator and the site personnel with administrative and detailed technical information that does not impact the safety of the subjects.

5.4. Outline of study procedures

Table 2 details study procedures to be conducted during the study.

Table 2 List of study procedures to be conducted at each survey visit

Epoch	Epoch 001	Epoch 002	Epoch 003	Epoch 004	Epoch 005	Epoch 006	Epoch 007	Epoch 008	Epoch 009	Epoch 010
Study Group[1]	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Survey 6**	**Survey 7**	**Survey 8**	**Survey 9**	**Survey 10**
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**
Informed consent	●	●	●	●	●	●	●	●	●	●
Check inclusion/exclusion criteria	●	●	●	●	●	●	●	●	●	●
Record demographic details	●	●	●	●	●	●	●	●	●	●
Record subject's vaccination status[2]	●	●	●	●	●	●	●	●	●	●
Record any anti-malarial medication or other medication received within the previous 14 days	●	●	●	●	●	●	●	●	●	●
Record relevant medical history (hospitalisation, reported fever, and care seeking behaviour)	●	●	●	●	●	●	●	●	●	●
Record axillary body temperature	●	●	●	●	●	●	●	●	●	●
Record malaria control measures used in the household	●	●	●	●	●	●	●	●	●	●
Record malaria prevention and risk factors	●	●	●	●	●	●	●	●	●	●
Take capillary blood sample[3]	●	●	●	●	●	●	●	●	●	●
Assessment of RDT[4]	●	●	●	●	●	●	●	●	●	●
Study conclusion	●	●	●	●	●	●	●	●	●	●

Footnotes to Table 2:

● is used to indicate a study procedure that requires documentation in the individual eCRF

[1] New sites will join the study after survey 3, depending on when they are included in EPI-MAL-002/-003. The total surveys at these new sites will therefore be less than 10.

[2] RTS,S/AS01 and 3rd dose DTP/HepB/Hib and first dose measles EPI vaccinations, only

[3] Capillary blood sample for determination of parasite prevalence (blood slides and NAAT by filter paper)

[4] In the event of measured fever at time of visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours or other symptoms/signs of clinical malaria, a rapid diagnostic test (RDT) will be conducted using capillary blood sample taken for blood slide preparation.

Table 3 List of study procedures to be conducted during survey

Epoch	Epoch 001	Epoch 002	Epoch 003	Epoch 004	Epoch 005	Epoch 006	Epoch 007	Epoch 008	Epoch 009	Epoch 010
Study Group	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Survey 6**	**Survey 7**	**Survey 8**	**Survey 9**	**Survey 10**
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**
Site specific information collection - Meteorological data[1]	●	●	●	●	●	●	●	●	●	●
Site specific information collection – Malaria Control Programme	●	●	●	●	●	●	●	●	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.

[1] Availability of meteorological data will be based on the site having the weather station. All sites received weather stations either post Survey 1 or 2 or at study start for the new sites.

5.5. Detailed description of study procedures to be conducted at each survey visit

5.5.1. Check inclusion and exclusion criteria

Check all applicable inclusion and exclusion criteria as described in Sections 4.2 and 4.3 before enrolment.

5.5.2. Informed consent

The signed/witnessed/thumbprinted informed consent of the subject's parent(s)/LAR(s) must be obtained before study participation. The signed informed assent of a subject below the age of consent (i.e., minor) should be obtained in addition to the signed informed consent by his/her parent(s)/LAR(s) according to local rules and regulations. Refer to Section 5.1 for the requirements on how to obtain informed consent and assent, as appropriate.

5.5.3. Collect demographic data

Record demographic data (date of birth, gender) and study area identification in the subject's eCRF.

5.5.4. Check and record medications/vaccinations

Record RTS,S/AS01$_E$ (all doses) and other vaccinations (i.e. 3rd dose DTP/HepB/Hib and first dose measles EPI vaccinations) administered, including dates. The vaccination status will be recorded in the eCRF.

Record use of any anti-malarial or any other medication within 14 days prior to study visit. Data to be verified from health card or medical prescription document.

5.5.5. Check and record relevant medical history

Record details of relevant medical history in the eCRF:

- Obtain reported history of temperature within the last 24 hours by interview
- Obtain details for hospitalisation for malaria within the last 3 months by interview and/or review of subject's health card
- Obtain details of visits to health provider for fever or malaria treatment in the previous 14 days by interview and/or review of subject's health card.

5.5.6. Assessment of body temperature

The following information must be recorded in the eCRF:

- Axillary temperature as measured by a digital thermometer at the time of the survey.

5.5.7. Collect data on malaria control interventions

Record the following malaria control measures in the subject's eCRF:

- Indoor residual spraying (IRS), mosquito net usage (including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN]), seasonal malaria chemoprevention (SMC), intermittent preventive treatment in infants (IPTi), and all anti-malaria therapy received within the last 14 days.

5.5.8. Collect data on malaria prevention measures and risk factors

Record the following information in the subject's eCRF:

- Repellents and local herbs used, rural/urban area, construction material for the house, floor, and roof, type of eaves (open/closed), use of electricity, and water source (distance from and type).

5.5.9. Capillary blood sampling

A capillary blood sample collected by finger/heel prick, will be taken during the study visit for the assessment of parasitaemia (see Section 5.6.2). The following biological samples will be taken at each survey visit:

- Blood for asexual and sexual (gametocyte) microscopy (2 slides, approximately 2 drops on each microscope slide).

  Preparation of microscopic slides in the field or at the laboratory is at the discretion of the study site staff. For microscopic slide preparation in the field, approximately 2 drops of blood will be applied directly on each microscopy slide. For microscopic slide preparation in the laboratory, the blood sample will be collected in provided microtubes for transport to the laboratory.

- Blood for asexual and sexual (gametocyte) NAATs (approximately 2 drops on filter paper).

In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours or other symptoms/signs of clinical malaria, a RDT will also be conducted using the capillary blood sample

Refer to the Module on Biospecimen Management in the SPM for general handling of blood samples.

5.5.10. Site specific information collection

Centre specific information about meteorological data such as rainfall, temperature and humidity, if available, will be collected by questionnaire and recorded in a separate database to that for subject-specific data. All sites received weather stations either post Survey 1 or 2 or at study start for the new sites.

Centre specific information about interventions from the malaria control programme in the study area, such as bednet usage (including ITN and LLIN), IRS, ACT, SMC, IPTp and IPTi, will be collected by questionnaire and recorded in a separate database to that for subject-specific data.

5.5.11. Recording of SAEs

Capillary blood sampling is the only invasive procedure involved in this study. SAEs related to this procedure will be recorded.

- Refer to Section 6.2 for procedures for the investigator to record SAEs. Refer to Section 6.3 for guidelines on how to submit SAE reports to GSK Biologicals.
- The subjects' parent(s)/LAR(s) will be instructed to contact the investigator immediately should the subjects manifest any signs or symptoms they perceive as serious.

5.5.12. Study conclusion

The investigator will:

- Review all the data collected to ensure accuracy and completeness
- Complete the Study Conclusion screen in the eCRF.

5.6. Biological sample handling and analysis

Please refer to the SPM for details of biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subjects but will be coded with the identification number for the subject (subject number).

- Collected samples will be used for protocol mandated research. In addition, these samples may be used to perform research related to the improvement, development and quality assurance of the laboratory tests described in this protocol. This may include the management of the quality of these tests, the maintenance or improvement of these tests, the development of new test methods, as well as making sure that new tests are comparable to previous methods and work reliably.
- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed will be invited to give another specific consent when signing the Informed Consent Form to allow GSK or a contracted partner to use the samples for future research including development of tests and their quality assurance. Future research will be subject to the laws and regulations in the respective countries and will only be performed once an independent Ethics Committee or Review Board has approved this research.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the subject's parent(s)/LAR(s).

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit/contact), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

5.6.1. Use of specified study materials

When materials are provided by GSK Biologicals, it is MANDATORY that all samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 7.3 for the definition of study cohorts/data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when GSK Biologicals does not provide material for collecting and storing samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM.

5.6.2. Biological samples

Table 4 Biological samples

Sample type	Quantity	Timepoint
Whole blood	Approximately 1 drop (RDT assessment)*	Survey Visit
Whole blood	Approximately 4 drops; 1 drop for the thin smear and up to 3 drops for the thick smear (merozoite and gametocyte microscopy assessment)	Survey Visit
Whole blood	2 to 3 drops; filter paper collection (parasite and gametocyte NAAT assessment)	Survey Visit

RDT = Rapid Diagnostic Test
NAAT = Nucleic Acid Amplification Test
*In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours or other symptoms/signs of clinical malaria.

5.6.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

In the event of fever measured at time of visit (axillary temperature ≥37.5°C) or reported fever in last 24 hours or other symptoms/signs of clinical malaria, a RDT will be conducted. The clinical management of subjects presenting with malaria as identified by

RDT or microscopy will follow National guidelines. Subjects will receive appropriate care according to standard clinical practice.

All blood slides with thick and thin smears will be made in duplicate. One slide with thick and thin smears will be sent for storage to a GSK dedicated laboratory facility and the remaining slide will be stored at the study site. For each slide, parasitaemia will be determined independently by two readers and in the case of non-concordance an additional read by a third independent reader will be carried out. Since there will be no full blood count performed in the study subjects the used methodology for the determination of *P. falciparum* asexual parasite density may be based on an assumed white cell count of 8000/µL. Please refer to APPENDIX A for a description of other methodologies for the determination of *P. falciparum* asexual parasite density.

Exploratory NAATs will be used to assess *P. falciparum* asexual prevalence and level of gametocyte parasitaemia (Table 5). Finger prick blood samples will be dried on protein saver card 903 (Whatman). Firstly real-time quantitative polymerase chain reaction (QT-PCR) is conducted for detection of asexual parasite DNA. If the results are positive, gametocyte detection by real-time quantitative nucleic acid sequence-based amplification (QT-NASBA) on messenger RNA will be performed. To ensure the integrity of the samples with respect to possible degradation of DNA/RNA, and therefore recording of false negative results, β2 microglobuline and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) control genes will be measured in a subset of samples for internal quality control purposes.

Table 5 Molecular Biology (NAAT tests)

System	Component	Method	Unit	Laboratory*
3667	18S parasite DNA	QT-PCR *Plasmodium falciparum*	Semi-quantitative results (e.g. H, M, L)	AMC
3668	Pfs25 gametocyte mRNA	QT-NASBA gametocyte	Qualitative	AMC
3669	GAPDH parasite DNA	PCR GAPDH	Positive/Negative	AMC
3670	β2 microglobuline mRNA	RT-PCR β2 microglobuline	Positive/Negative	AMC

*Refer to APPENDIX B for the laboratory addresses
QT-PCR: quantitative polymerase chain reaction
QT-NASBA: quantitative nucleic acid sequence-based amplification
GAPDH: glyceraldehyde 3-phosphate dehydrogenase
RT-PCR: reverse transcriptase polymerase chain reaction
AMC: Academic Medical Center

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

6. SAFETY

The procedure of capillary blood sampling will be performed once at the survey visit. Only SAEs related to the blood sampling procedure that occur in temporal association with the procedure will be recorded (up to 7 days after the survey visit).

The investigator or site staff is/are responsible during the study for the detection and documentation of events meeting the criteria and definition of a SAE as provided in this protocol.

Each subject's parent(s)/ LAR(s) will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

6.1. Safety definitions

6.1.1. Definition of a serious adverse event

An SAE is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   NB: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalisation or prolongation of an existing hospitalisation,

   NB: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting.

   Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

   Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an SAE.

d. Results in disability/incapacity,

   NB: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g., sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

   Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation.

6.2. Detecting and recording SAEs

6.2.1. Time periods for detecting and recording SAEs

In order to fulfil international reporting obligations, SAEs that are related to study participation (i.e., protocol-mandated procedures, invasive tests, a change from existing therapy) will be collected and recorded from the time the subject consents to participate in the study/study start until she/he is discharged from the study.

6.2.2. Evaluation of SAEs

6.2.2.1. Active questioning to detect SAEs

When an SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding the SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/ or other clinical information. In such cases, the diagnosis should be documented as the SAE and not the individual signs/symptoms.

6.2.2.2. Assessment of the intensity of SAEs

The investigator will assess the maximum intensity that occurred over the duration of the event for all SAEs recorded during the study. The assessment will be based on the investigator's clinical judgement.

The intensity should be assigned to one of the following categories:

1 (mild)	=	An SAE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An SAE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An SAE which prevents normal, everyday activities (in a young child, such an AE would, for example, prevent attendance at school/ kindergarten/ a day-care centre and would cause the parent(s)/ LAR(s) to seek medical advice.)

An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 6.1.1.

6.2.2.3. Assessment of causality

The investigator should assess the causality of each SAE. The investigator will use clinical judgement to determine the relationship between the SAEs and study participation. Alternative causes, such as natural history of the underlying diseases, other concomitant therapy and other risk factors will be considered and investigated.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly.

If an event meets the criteria to be considered as 'serious' (see Section 6.1.1), additional examinations/tests will be performed by the investigator in order to determine ALL possibly contributing factors to each SAE.

Possibly contributing factors include:

- Medical history.
- Concomitant medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Other cause (specify).

6.2.2.4. Assessment of outcomes

The investigator will assess the outcome of all SAEs recorded during the study as:

- Recovered/ resolved.
- Recovering/ resolving.
- Not recovered/ not resolved.
- Recovered with sequelae/ resolved with sequelae.
- Fatal.

6.3. Reporting of SAEs

6.3.1. Prompt reporting of SAEs related to study participation to GSK

SAEs that occur in the time period defined in Section 6.2.1 will be reported promptly to GSK within the timeframes described in Table 6 once the investigator determines that the event meets the protocol definition of an SAE.

Table 6 Timeframes for submitting SAEs related to study participation to GSK

Type of event	Initial reports		Follow-up of relevant information on a previous report	
	Timeframe	Documents	Timeframe	Documents
SAEs related to study participation	24 hours*	Electronic SAE report	24 hours*	Electronic SAE report

* Timeframe allowed after receipt or awareness of the information.

6.3.2. Contact information for reporting SAEs to GSK

Study Contact for Reporting SAEs
Refer to the local study contact information document.
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Email: PPD Fax: PPD or PPD

6.3.3. Completion and transmission of SAEs reports related to study participation to GSK

Once an investigator becomes aware that an SAE has occurred in a study subject, the investigator (or designee) must complete the information in the electronic Expedited Adverse Event Report WITHIN 24 HOURS. The report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding an SAE, the report should still be completed within 24 hours. Once additional information is received, the report should be updated WITHIN 24 HOURS.

The investigator will always provide an assessment of causality at the time of the initial report.

6.3.3.1. Back-up system in case the electronic reporting system does not work

If the electronic reporting system does not work, the investigator (or designee) must complete, then date and sign a paper Expedited Adverse Event Report and fax it to the GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic reporting system is not working and NOT if the system is slow. As soon as the electronic reporting system is working again, the investigator (or designee) must complete the electronic Expedited Adverse Event Report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic reporting system.

6.3.4. Updating of SAE information after freezing of the subject's eCRF

When additional SAE information is received after freezing of the subject's eCRF, new or updated information should be recorded on a paper Expedited Adverse Event Report, with all changes signed and dated by the investigator. The updated report should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (see the Sponsor Information) within the designated reporting time frames specified in Table 6.

6.3.5. Regulatory reporting requirements for SAEs

The investigator will promptly report all SAEs to GSK Biologicals in accordance with the procedures detailed in Section 6.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under epidemiological investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

6.4. Follow-up of SAEs

6.4.1. Follow-up during the study

After the initial SAE report, the investigator is required to proactively follow each subject and provide further relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs, refer to Table 6).

All SAEs documented at a previous visit/ contact and recorded as not recovered/ not resolved or recovering/ resolving will be reviewed at subsequent visits/ contacts until the end of the study.

6.4.1.1. Follow-up after the subject is discharged from the study

The investigator will follow-up subjects:

- With SAEs until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/ she will provide this information to GSK Biologicals using a paper Expedited Adverse Event Report.

GSK Biologicals may request that the investigator performs or arranges for the conduct of additional clinical examinations/ tests and/ or evaluations to elucidate as fully as possible the nature and/ or causality of the SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

7. STATISTICAL METHODS

7.1. Endpoints

7.1.1. Primary endpoints

Primary

- Occurrence of *P. falciparum* parasitaemia (using microscopy)

  Criteria/definitions: infection with *P. falciparum* determined using a blood smear slide and determined using microscopy.

- Occurrence of malaria control interventions

  Criteria/definitions: malaria control interventions are mosquito net usage (including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN]), indoor residual spraying (IRS), seasonal malaria chemoprevention (SMC), intermittent preventative treatment in infants (IPTi), and ACT therapy received within the last 14 days.

7.1.2. Secondary endpoints

Secondary

- Demography and history characteristics

  Criteria/definitions: gender, age, medical history.

- Occurrence of *Plasmodium* species other than *P. falciparum* (using microscopy)

  Criteria/definitions: infection with *Plasmodium* species other than *P. falciparum* determined using a blood smear slide and microscopy.

- Occurrence of uptake and timing of the third dose of DTP/HepB/Hib and the first measles EPI vaccines

  Criteria/definitions: vaccination record of receipt of dose 3 of the DTP/HepB/Hib and the first dose of the measles EPI vaccines.

- Occurrence of anti-malarial therapy

  Criteria/definitions: any anti-malarial therapy received in the last 14 days.

- Occurrence of measured fever

  Criteria/definitions: any measured fever at time of visit (axillary temperature ≥37.5°C).

- Occurrence of reported fever

  Criteria/definitions: any reported fever occurring in the last 24 hours.

- Occurrence of care seeking behaviour

  Criteria/definitions: occurrence of visits to health providers following reported fever or malaria in the previous 14 days.

- Geo-referencing characteristics

  Criteria/definitions: positioning of the subject's residence will be attributed to a segment with a unique ID from the grid referencing study area map in which the subject resides, where necessary, grouping small geographically proximate villages so that each segment has at least 10 study subjects to avoid PII, and proceeding as far as geographically appropriate.

- Occurrence of individual malaria prevention measures and risk factors

  Criteria/definitions: malaria prevention measures are repellents and local herbs not specifically recommended by the national programme and risk factors are rural/urban area, construction material for the house, floor and roof, type of eaves (open/closed), use of electricity and water source (distance from and type).

7.1.3. Tertiary endpoints

- Occurrence of RTS,S/AS01$_E$ vaccine doses

  Criteria/definitions: vaccination record of each dose received of the RTS,S/AS01$_E$ vaccine.

- Occurrence of *P. falciparum* parasitaemia (using NAAT)

  Criteria/definitions: detection of *P. falciparum* on dried blood spot and determined using NAAT.

- Occurrence of sexual *P. falciparum* parasitaemia (using microscopy)

  Criteria/definitions: detection of sexual *P. falciparum* using a blood smear slide and determined using microscopy.

- Occurrence of sexual *P. falciparum* (using NAAT/QT-NASBA)

  Criteria/definitions: detection of sexual *P. falciparum* on dried blood spot and determined using NAAT.

7.2. Determination of sample size

In this study, sample sizes have been designed to ensure sufficiently narrow confidence intervals (CIs) around centre-wise parasite prevalence estimates (with a maximum residual standard error [RSE] of 0.25) according to the WHO definition [WHO, 1963] (2-9 years) and to the JTEG requested age (<5 years).

Moreover, when these estimates are used to adjust vaccine clinical malaria estimates from EPI-MAL-002/-003 for annual fluctuations in MTI and control measure use, a sufficient level of precision for the vaccine ineligible subgroup is maintained. In addition, the ability to detect longitudinal trends in parasite prevalence has been considered in these sample size calculations.

7.2.1. Children aged 6 months to <5 years

The sample size is computed for each centre separately and is considered for the point estimate of prevalence of infection among children aged at least 6 months and less than 5 years during one cross-sectional survey.

The effective sample size is computed to obtain an estimation of the prevalence of infection with a relative standard error equal to a maximum of 0.25.

Given that the expected sample size should be greater than 100, CI limits are computed using the approximated 95% CI.

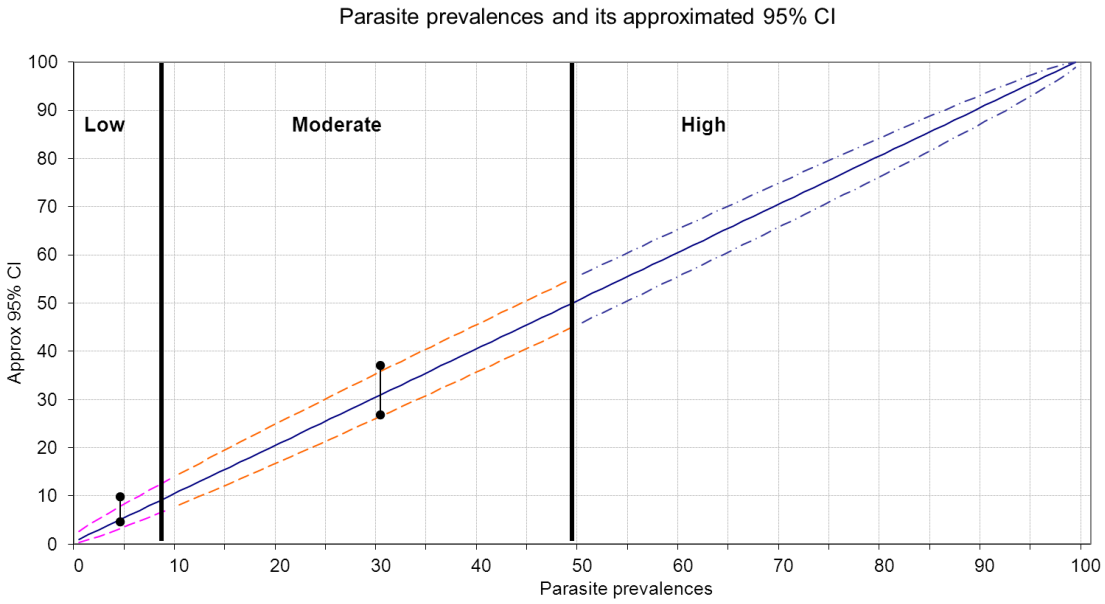
In Table 7 values of the CI limits are computed for several expected parasite prevalences in function of effective sample sizes equal to 100, 200, 300, 400, 600 and 1000 subjects. To meet the criteria of precision defined above, the effective sample sizes are 400 eligible subjects. Indeed, for an expected parasite prevalence of 5%, the RSE is equal to 0.23 (= half-width of CI / 2*p) (shaded values in Table 7).

Table 7 Approximated 95% CI limits around different values of observed parasite prevalence computed in function of several sample sizes

Sample size	Approx. 95% CI	Expected parasite prevalence										
		5	10	20	25	30	40	50	60	70	80	90
100	LL (%)	1.9	5.2	12.9	17.1	21.5	30.5	39.9	49.7	59.9	70.6	82.0
	UL (%)	11.8	18.0	29.4	34.8	40.1	50.3	60.1	69.5	78.5	87.1	94.8
200	LL (%)	2.6	6.4	14.8	19.3	23.8	33.2	42.9	52.8	63.1	73.6	84.8
	UL (%)	9.3	15.2	26.4	31.7	36.9	47.2	57.1	66.8	76.2	85.2	93.6
300	LL (%)	2.9	7.0	15.7	20.3	24.9	34.5	44.2	54.2	64.4	74.9	85.9
	UL (%)	8.3	14.1	25.1	30.4	35.6	45.8	55.8	65.5	75.1	84.3	93.0
400	LL (%)	3.2	7.3	16.3	20.9	25.6	35.2	45.0	55.0	65.2	75.7	86.5
	UL (%)	7.7	13.5	24.3	29.6	34.8	45.0	55.0	64.8	74.4	83.7	92.7
600	LL (%)	3.5	7.8	16.9	21.6	26.4	36.1	45.9	55.9	66.1	76.5	87.2
	UL (%)	7.1	12.8	23.5	28.7	33.9	44.1	54.1	63.9	73.6	83.1	92.2
1000	LL (%)	3.8	8.2	17.6	22.4	27.2	37.0	46.9	56.9	67.0	77.4	87.9
	UL (%)	6.6	12.1	22.6	27.8	33.0	43.1	53.1	63.0	72.8	82.4	91.8

In Figure 1, precision around expected parasite prevalence is illustrated taking into account the defined sample size. For example, for an expected parasite prevalence of 7%, the lower and upper limits of the approximated 95% CI are equal to 4.8% and 10.1%, respectively. For an expected parasite prevalence of 31%, the limits are 25.9% and 36.6%, respectively.

Figure 1 Illustration of the lower (LL) and upper (UL) limits of the approximated 95%CI built around observed parasite prevalences for a sample of 400 subjects



Study sites are in different endemicity classes, so a sample of 400 subjects in this age class will allow for suitable confidence limits around parasite prevalence estimates for sites within each of the endemicity classes.

Note that with the sampling strategy using the proposed stratification by age (Section 4.1.2), 400 eligible subjects aged at least 6 months and less than 5 years will be recruited.

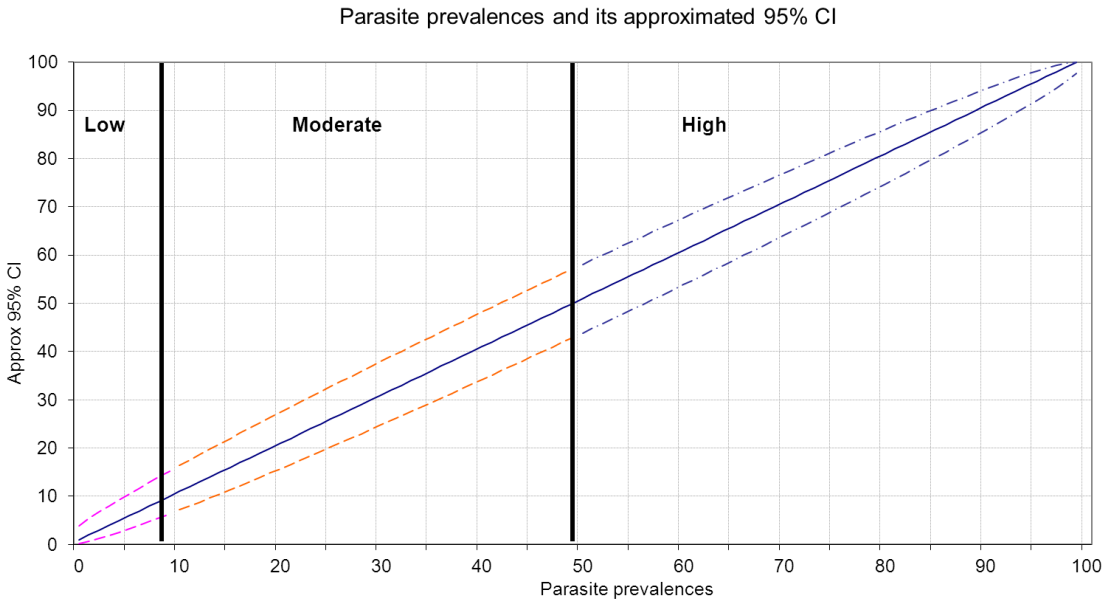
7.2.2. Children aged 2 to <10 years

Based on the defined recruitment scheme (see Section 4.1.1), the sample size of children aged 2 to <10 years will be approximately equal to the number of eligible subjects aged 0-5 years; ensuring the adequate precision for this age group. Indeed, following the recruitment scheme explained in Section 4.1.1, 420 subjects would be aged between 2 to <10 years.

7.2.3. Children aged 5 to <10 years

Concerning children aged at least 5 years and less than 10 years, the effective sample size is computed to obtain an estimation of the prevalence of infection with a relative standard error equal to a maximum of 0.35. Therefore, the sample size will be approximately 200 eligible subjects (see Figure 2).

Figure 2 Illustration of the lower (LL) and upper (UL) limits of the approximated 95%CI built around observed parasite prevalences for a sample of 200 subjects



Since the precision of 0.25 was adequate and determined for the WHO (children aged 6 months to <5 years) and JTEG (children aged 2 to <10 years) definitions, the sample size for the subgroup of children 5-9 years was fixed at 200 which gives a relative standard error equal to a maximum of 0.35. In addition, it is anticipated that children in this older age group are more likely to be at school and it may take longer to recruit this age group with subsequent logistics and financial implications.

7.3. Cohorts for analyses

7.3.1. Total cohort

The Total cohort will include all subjects enrolled in the study.

All the information for these subjects will be collected in the eCRF (after receiving signed informed consent).

7.3.2. According-To-Protocol cohort

The According to Protocol (ATP) cohort will include all evaluable subjects (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) for whom at least one laboratory result of the blood sample is available.

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

7.4. Derived and transformed data

- Age in the study will be computed as the difference between the date of informed consent and the date of birth. The age will be expressed in the following groups: 0.5 to <3 years, 3 to <5 years, 5 to <10 years.
- The fever status will be derived from the axillary temperature measured at the time of the visit. The value 'Yes' will be given if the temperature is ≥37.5°C.
- The parasite density will be grouped into the following classes:
  - Low: <2500 parasites/ µL
  - Medium: 2500 – 9999 parasites/ µL
  - High: 10000 – 19999 parasites/ µL
  - Very high: ≥20000 parasites/ µL

  In the case of two positive slides readings, the parasites density (parasites/µL) at the subject level will be defined as the geometric mean of both (those positive) slide reading values if the subject status is defined as positive. In the case of three positive slide readings, the two closest readings will be selected to calculate the geometric mean. In the case of negative parasite status, the density will be recorded as missing.

7.5. Analysis of demographics

The number of subjects enrolled as well as the number excluded from the ATP analyses will be presented. Demographic characteristics (age and gender) will be summarised using descriptive statistics.

Centre specific data regarding the malaria control programme will be summarised for each site and cross-surveys separately.

7.6. Analysis of primary objectives

The parasite prevalence will be estimated as the proportion of subjects infected divided by the number of subjects tested. All CIs will be two-sided 95% CIs computed using the exact method, calculated from Proc StatXact [Clopper, 1934]. The estimates of parasite prevalence will be done each year and for each site separately.

The estimates of the use of malaria control measures will be estimated the same way; as the proportions of subjects using malaria control measures divided by the number of subjects for which this information is available.

7.7. Analysis of secondary objectives

7.7.1. Trends in parasite prevalence

7.7.1.1. Overall

First the trends in parasite prevalence between each cross-sectional malariometric surveys will be tested using the Cochran-Armitage trend test [Agresti, 1990]. This hypothesis test will be performed on the parasite prevalence computed on the independent samples of subjects in each survey separately within each site.

7.7.1.2. By vaccine eligible/ineligible subgroup: Statistical analyses for vaccine association adjustments and association between vaccine and transmission

For the analysis of association between RTS,S/AS01$_E$ vaccine and transmission, two subgroups must be considered:

- Vaccine eligible subgroup (see glossary of terms for definition). This subgroup will be used to assess the association between vaccination and transmission intensity, assuming a constant MTI in unvaccinated subjects (which will be measured using the second subgroup).
- Vaccine ineligible subgroup (see glossary of terms for definition). Due to the fact that the main reservoir of malaria is in the population between 5-20 years old (based on data collected in parallel [Epi-Mal-001 study] to the pivotal efficacy Phase III trial, not published) and MTI will be assessed for approximately 5 years following the introduction of the vaccine, it will be assumed that there will be negligible herd immunity effect. Therefore, this subgroup will be used to detect any annual fluctuation (control group). If there is any herd effect, then the vaccine effect will be an underestimate.

7.7.1.2.1. Annual fluctuations: vaccine ineligible subgroup

Once the cross-surveys are collected, the independent samples of subjects in each survey will be tested and assessed using a Chi-square statistic to test the significance of the differences among the parasite prevalences in each survey; meaning annual fluctuations.

If a significant difference is detected, further analyses will be done to characterise the difference(s).

Two methods will be then used. The first method is by two-by-two comparison between each cross-survey result. The advantage is identification of non-linear variations. The disadvantage is that longitudinal aspects of all surveys are not taken into consideration.

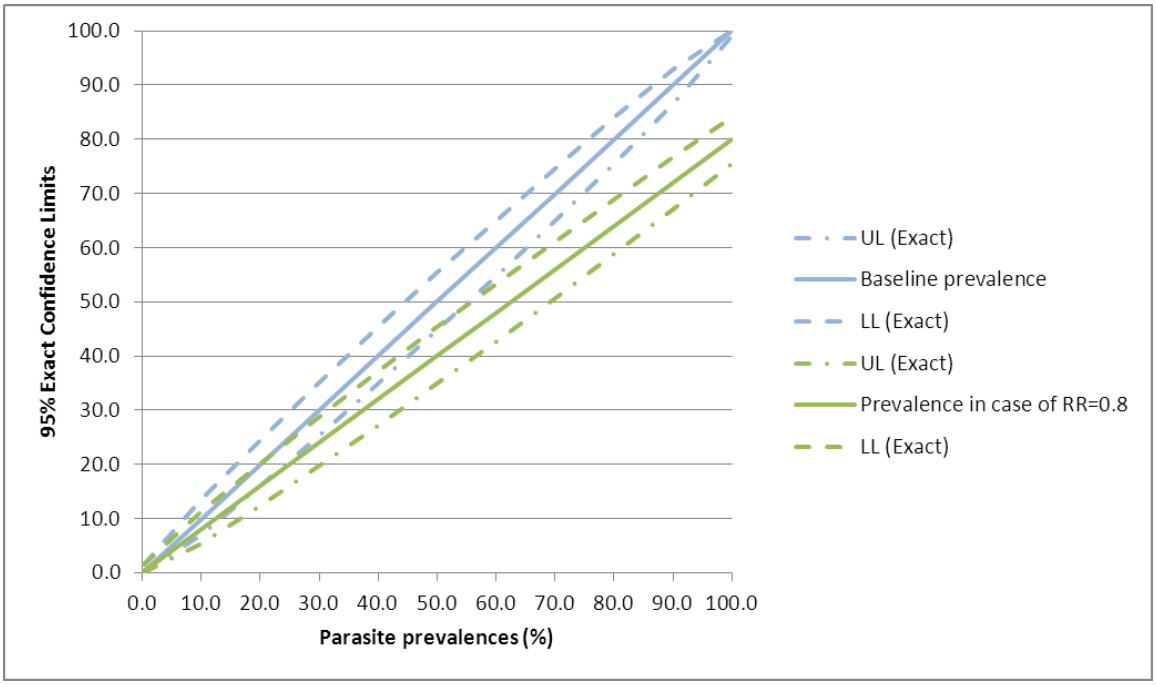
As shown in Table 8 and in Figure 3, a relative risk (RR) of one year of the study to another equal to 0.8 and 0.6 could be detected for a baseline prevalence greater than 60% and 20%, respectively. Note; these statements are based on non-overlapping CIs.

Table 8 Power estimation to detect a certain RR (from 0.1 to 0.9) depending on different baseline prevalences (from 0.1 to 0.9) with a sample size of 360 subjects

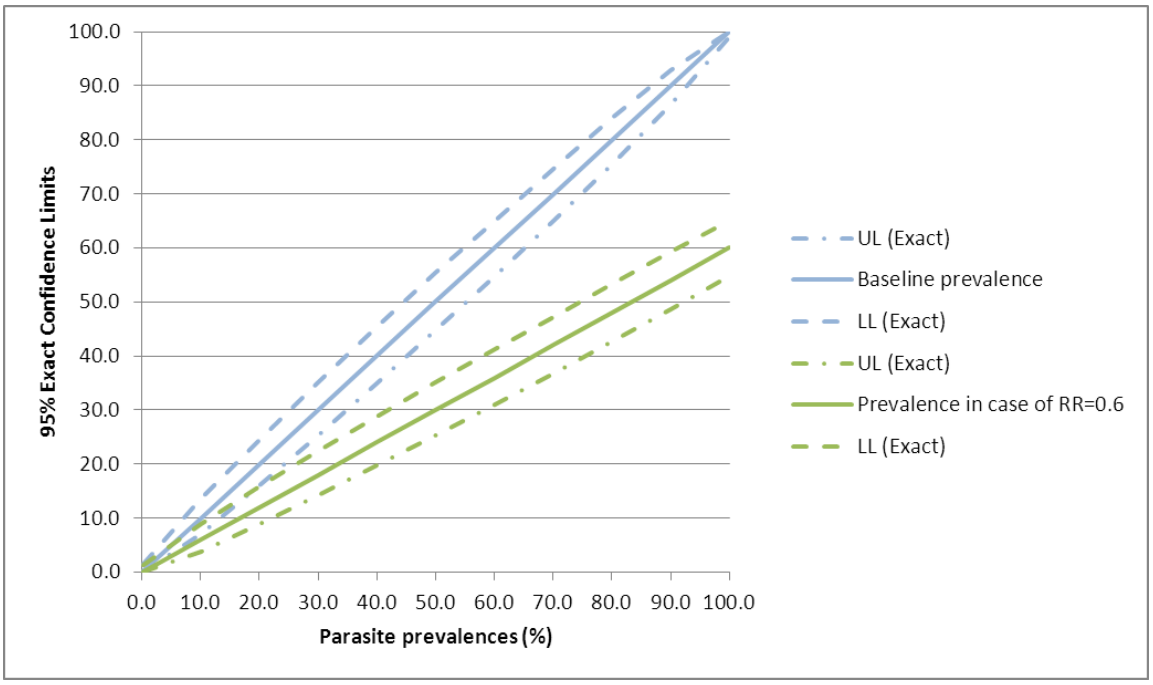
	Relative Risk								
Baseline	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
0.1	1	1	0.97	0.89	*0.72*	*0.51*	*0.3*	*0.15*	*0.07*
0.2	1	1	1	1	0.97	0.83	*0.57*	*0.29*	*0.1*
0.3	1	1	1	1	1	0.97	0.79	*0.44*	*0.14*
0.4	1	1	1	1	1	1	0.93	*0.61*	*0.2*
0.5	1	1	1	1	1	1	0.98	*0.77*	*0.27*
0.6	1	1	1	1	1	1	1	0.9	*0.37*
0.7	1	1	1	1	1	1	1	0.97	*0.51*
0.8	1	1	1	1	1	1	1	1	*0.71*
0.9	1	1	1	1	1	1	1	1	0.93

Figure 3 Illustration of the lower (LL) and upper (UL) limits of the exact 95% CI built around both compared prevalences with a sample size of 360 subjects

a. based on a RR of 0.8



b. based on a RR of 0.6



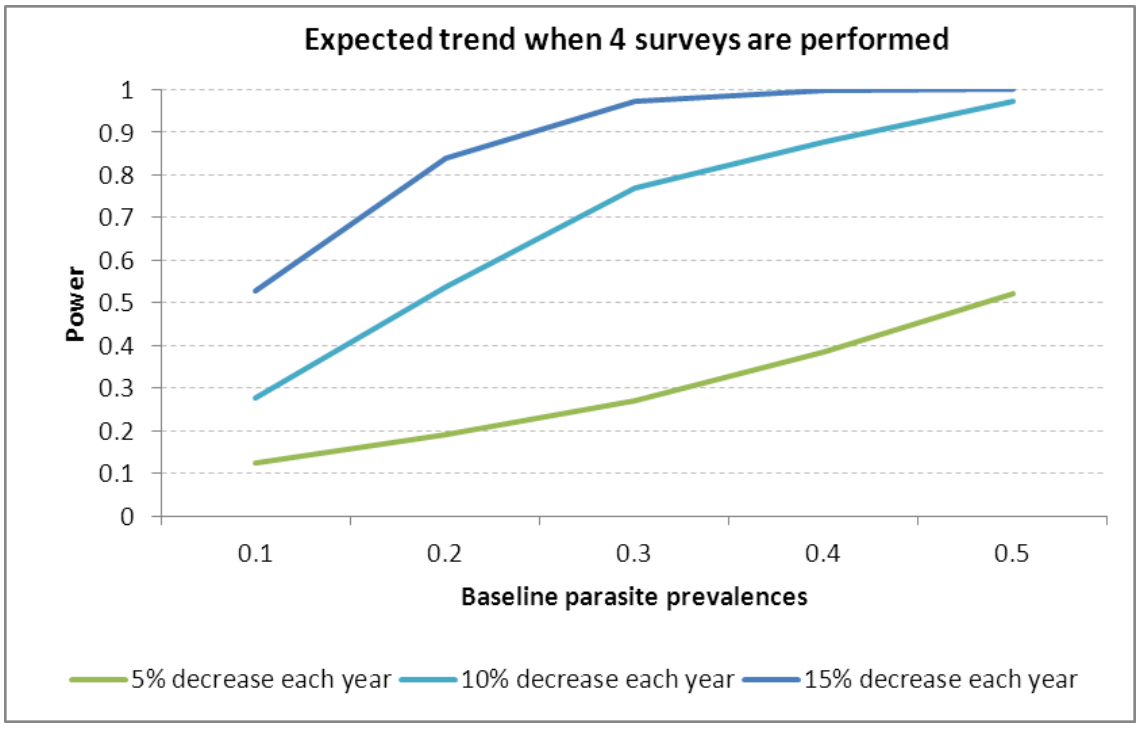
The second method is to perform a Cochran linear hypothesis trend test in which all longitudinal estimations will be used to detect a linear trend. However, only linear trends could be detected. As shown in Figure 4, a 5% decrease could not be detected if the

baseline incidence is smaller than 50%. Based on 4 surveys, and a threshold of 90% power, a 10% decrease could be detected for a baseline incidence higher than or equal to 40% and a 15% decrease could be detected for a baseline incidence higher than or equal to~25%. The power improves with an increased number of surveys.

Table 9 Power estimation to detect a certain RR (from 0.1 to 0.9) depending on different baseline prevalence with a sample size of 240 subjects

	Relative Risk								
Baseline	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
0.1	0.99	0.96	0.88	*0.73*	*0.55*	*0.36*	*0.22*	*0.12*	*0.06*
0.2	1	1	1	0.97	0.87	*0.67*	*0.42*	*0.21*	*0.08*
0.3	1	1	1	1	0.98	0.87	*0.62*	*0.32*	*0.11*
0.4	1	1	1	1	1	0.97	0.79	*0.45*	*0.14*
0.5	1	1	1	1	1	0.99	0.92	*0.6*	*0.19*
0.6	1	1	1	1	1	1	0.98	*0.75*	*0.26*
0.7	1	1	1	1	1	1	1	0.89	*0.37*
0.8	1	1	1	1	1	1	1	0.98	*0.54*
0.9	1	1	1	1	1	1	1	1	0.8

Figure 4 Illustration of the power to detect a trend using the Cochran trend test, and based on a sample size of 360 subjects and an assumed decrease each year of 5%, 10% or 15%

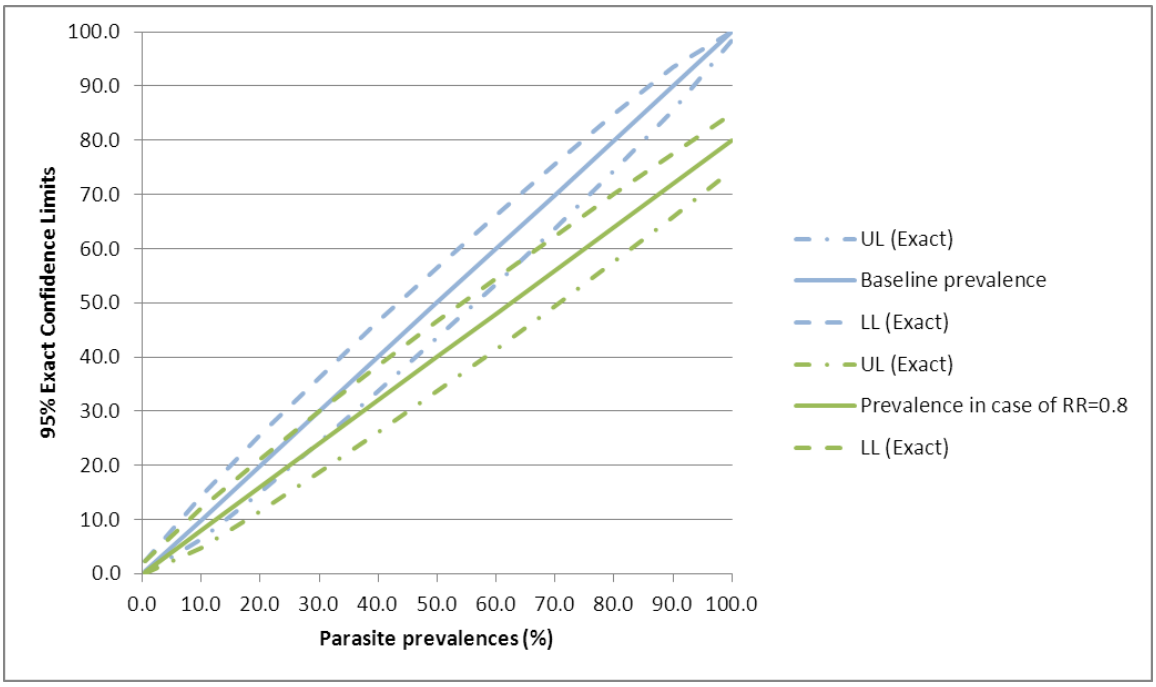


7.7.1.2.2. Association between vaccine and parasite prevalence: vaccine eligible subgroup

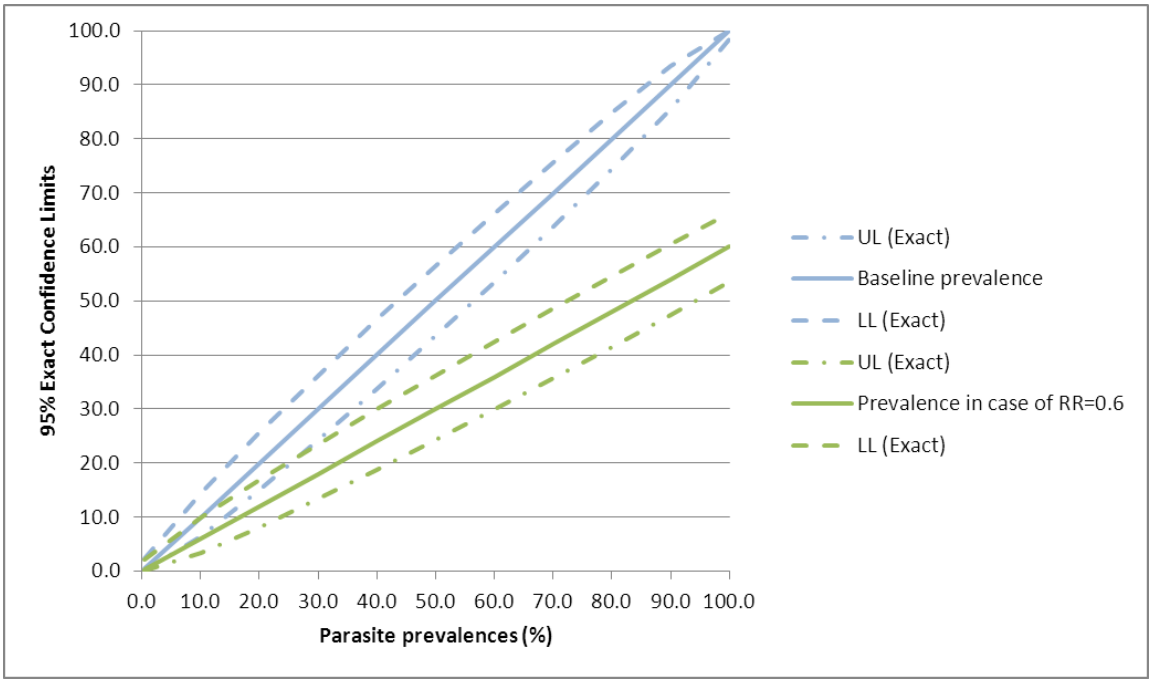
Using the same first methodology for the vaccine ineligible subgroup (Section 7.7.1.2.1), a statistically significant difference could be detected for a RR of 0.8 and 0.6 at a baseline prevalence greater than 70% and 30%, respectively, as shown in Figure 5 or in Table 9.

Figure 5 Illustration of the lower (LL) and upper (UL) limits of the exact 95% CI built around both compared prevalences with a sample size of 240 subjects

a. based on a RR of 0.8



b. based on a RR of 0.6



7.7.2. Other prevalences and proportions

The parasite prevalence and the estimates of the use of malaria control measures will be estimated by age group as defined in Section 7.4.

The prevalence of *Plasmodium* species other than *P. falciparum* will be estimated as the proportion of subjects infected divided by the number of subjects tested.

Prevalence for each segment will be estimated using the following criteria for level of endemicity: 'high' ≥ 60%, 'moderate' 20%-59%, and 'low' <20% relating to the proportion of infected children in each segment [Snow, 1997; Omumbo, 1998].

The proportion of subjects having received the third dose of DTP/HepB/Hib and the first dose of measles EPI vaccine will be estimated as the ratio of the subjects having received the third dose of DTP/HepB/Hib and the first dose of measles EPI vaccine divided by the number of subjects who should have received the third dose of DTP/pentavalent and the first dose of measles EPI (i.e. 14 weeks and 9 months of age, respectively). Moreover, among the subjects having received the third dose of DTP/HepB/Hib and the first dose of measles EPI vaccine, the proportions of subjects having received them on time, in advance and with delay will be estimated.

The care seeking behaviours will be described as the proportions of subjects having received care among all subjects with reported fever or malaria in the previous 14 days by the place they sought for care.

The prevalence of gametocyte presence will be estimated as the proportion of subjects infected divided by the number of subjects tested.

The parasite and gametocyte prevalences will also be estimated separately for microscopy and NAAT results.

7.7.3. Within-site geographical heterogeneity and risk factors

To assess within-site geographical heterogeneity, the parasite prevalence will be estimated separately in each village, grouping villages where necessary. Moreover, these geo-referencing characteristics will be part of the risk factor analysis described hereafter.

To describe the risk factors for malaria, a multivariable logistic regression will be used with all potential confounding variables.

7.8. Analysis of tertiary objectives

The comparisons of asexual and sexual (gametocyte) parasitaemia measured by microscopy and NAAT will be done using Cohen's Kappa coefficient. The hypothesis test will be done per centre, and overall.

7.9. Interpretation of analyses

Comparative analyses will be descriptive with the aim to characterise the difference between groups in the endpoint(s) related to the objective(s). These descriptive analyses should not be interpreted.

7.10. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

7.10.1. Sequence of analyses

The analysis will be performed in each epoch, on as clean as possible data for the initial epochs and on clean data for the final epoch. The objective assessing trends will be analysed only at the final epoch. The other objectives will be analysed at all epochs.

All analyses, apart from the trend analyses, will be done for each epoch by centre and by classes of age (6 months to <5 years versus 5 to <10 years and 6 months to <3 years versus 3 to <10 years), and by year for the age group 6 months to <5 years. They will be detailed in the Statistical Analysis Plan (SAP) and the Tables, Figures, Listings (TFL) before the analysis. Statistical reports will be written after each epoch. A clinical report will be written after Surveys 2 and 5 and an integrated report will be written at the end of the study.

7.10.2. Statistical considerations for interim analyses

Only descriptive analyses and estimations of point estimate prevalence will be computed for each interim analysis. These analyses will be descriptive only. Therefore no adjustment of type I error is needed.

Concerning the sample size required for each interim analysis, please refer to Section 7.2.

8. ADMINISTRATIVE MATTERS

To comply with ICH GCP or other applicable guidelines administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality, ownership and publications must be met.

8.1. Electronic Case Report Form instructions

A validated GSK defined electronic data collection tool will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a GSK Biologicals' Site Monitor at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

Once the database is archived and the clinical study report is complete and approved by all parties, each participating investigator will be provided with a CD-ROM of the final version of the data generated at his/her investigational site.

8.2. Study monitoring by GSK Biologicals

GSK will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform an eCRF review and a Source Document Verification (SDV). By SDV we understand verifying eCRF entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the eCRF. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the eCRF will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For eCRF, the monitor will mark completed and approved screens at each visit.

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and GSK procedures.

8.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g., audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g., microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

GSK will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP or other applicable guidelines, any institutional requirements or applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify GSK of any changes in the archival arrangements, including, but not limited to, archival at an off-site facility and transfer of ownership of the records in the event the investigator leaves the site.

8.4. Quality assurance

To ensure compliance with GCP or other applicable guidelines and all applicable regulatory requirements, GSK may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

8.5. Posting of information on publicly available registers and publication policy

Study information from this protocol will be posted on public registers before enrolment of subjects begins.

Interventional studies that do not evaluate vaccines/products are progressed for publication in the scientific literature when the results provide important scientific or medical knowledge.

8.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK Biologicals site or other mutually-agreed location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

9. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

10. REFERENCES

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APPENDIX A LABORATORY ASSAYS - DETERMINATION OF PARASITAEMIA BY MICROSCOPY

Slide reading methodology

All slide readers will be required to demonstrate proficiency at "Competent" level or above as documented by certification/training records [World Health Organization, 2009].

For further details of the reading methodology described, refer to the WHO Basic Malaria Microscopy Learners Guide [World Health Organization, 1991].

Slide preparation and assessing parasite presence

Slide preparation: During subject visits, capillary blood samples are used to produce two slides per subject containing both a thick and thin blood smear per slide. The thin film is produced from one small blood drop in the centre of the slide. Using the edge of a second, clean slide, firmly spread the single drop of blood across the sample slide. The thick film is produced from 3 larger drops of blood approximately 1cm away from the thin film. The drops should be joined and spread using the corner of the spreader to become a circular thick film of approximately 1cm diameter.

Thick film examination: A 100-field examination of the thick film should be conducted to assess presence of parasites and species.

Negative result: 100 fields free of parasites are read before a slide is declared negative.

Positive result: If parasites are present within reading of 100 fields, the slide is positive. Positive slides are examined for a further 100 fields to ensure all species present are detected.

Parasite density counting against assumed 8000 leukocytes per microlitre

In this method of estimating parasite density, it is assumed that there are 8000 leukocytes per microlitre of blood.

Step 1: Use 2 tally counters, one to count parasites and the other to count leukocytes.

a. If upon counting 200 leukocytes, 10 or more parasites have been counted, the results can be recorded as the parasites per 200 leukocytes.

b. If upon counting 200 leukocytes, 9 or fewer parasites have been recorded, the reader should continue counting until 500 leukocytes have been counted and the number of parasites per 500 leukocytes can be recorded.

Step 2: Convert the number of parasites relative to leukocyte count to an estimated number of parasites per microlitre:

$$\frac{\text{number of parasites x 8000}}{\text{number of leukocytes}} = \text{parasites per microlitre}$$

For example, if 20 parasites were observed upon counting 500 leukocytes, it would give an estimated 320 parasites per microlitre.

It should be noted that the count should be by species, and counts for *P. falciparum* should be made for both gametocytes and asexual parasites.

Criteria for concordance for double reading of slides

All slides will be read twice, by two independent readers to quantify the *P. falciparum* parasite presence and density. If slides are judged to be discordant, a third independent read will be organised in the following cases:

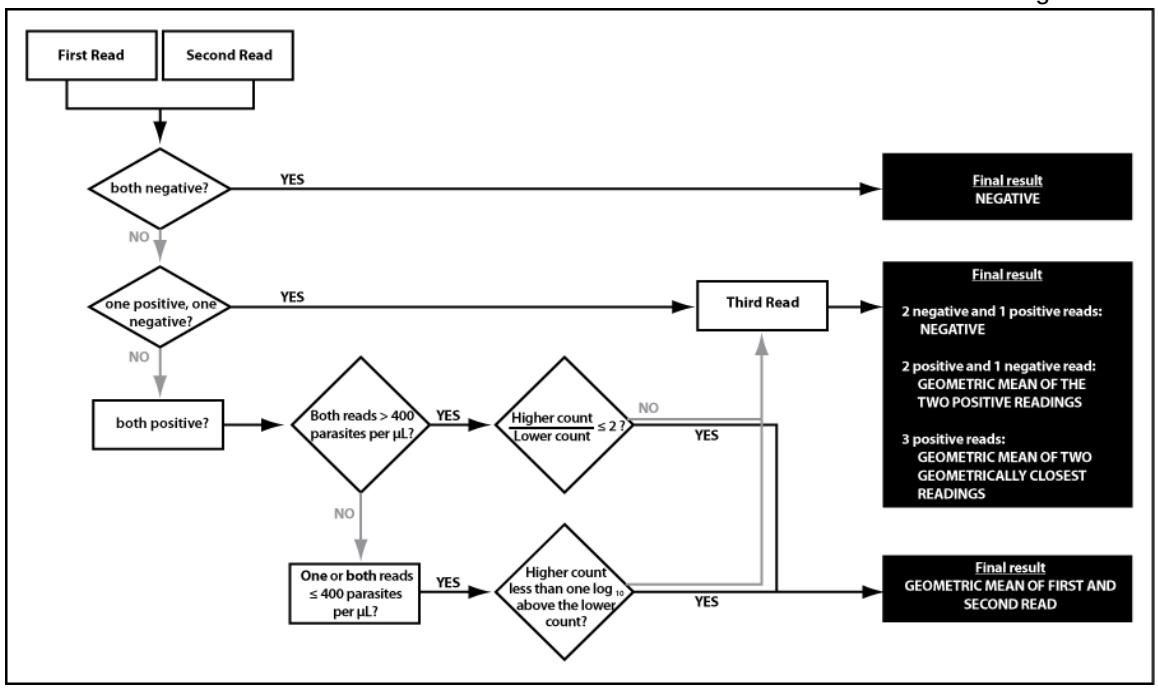
- The result from one reader is negative and the one of the other is positive.
- For high and medium positive parasitaemia results (blood parasitaemia >400/μL), the higher count divided by the lower count is >2.
- For low parasitaemia (blood parasitaemia ≤400/μL), the highest reading density is more than one $\log_{10}$ higher than the lowest reading.
- If parasitaemia result is high or medium in one slide and the result from the other slide reading is low, i.e. one is >400/μL and the other is ≤400/μL, criterion (C) will be applied.

Determination of final result

If there are two concordant results the final result is the geometric mean of the two readings.

If the first two readings are discordant then the final result will follow the following principles:

- For cases of positive/negative discrepancy (A), the majority decision will be adopted. If the decision is positive, the final result will be the geometrical mean of the two positives.
- For cases of three positive reads (B and C), the final result will be the geometric mean of the two geometrically closest readings.



Identification of *Plasmodium* species

Positive parasitaemia identified on any thick blood film must always be identified to species. This will be done on thin blood film except in case of low parasitaemia.

Archiving of blood slides

Two blood slides are taken, one for reading and archiving at the centres; the second one will be archived centrally at a GSK dedicated laboratory facility.

DETERMINATION OF PARASITAEMIA BY NAAT

Q-PCR

The accurate quantification of *Plasmodium falciparum* parasite numbers by real time Q-PCR is an important tool for monitoring growth kinetics and estimating the parasite density in blood from a subject.

The real time Q-PCR assay developed by AMC-BR targets the 18S gene of the *Plasmodium falciparum*. The final output of the assay is semi-quantitative (e.g. High, Medium, Low) because it is not possible to know exactly how much blood is extracted from the filter paper. Real-time PCR technology allows the determination of the copy number of sequence-specific nucleic acid target molecules. The amplification of the target sequence is detected using the 5’ nuclease assay based on the Taqman chemistry. In those PCR experiments, the formation of a PCR product is monitored in real-time during amplification by means of a fluorogenic probe that binds specifically to the amplified product. The reporter fluorophore is at the 5’ end of the Taqman probe and the quencher is at the 3’ end. As long as the probe is intact, no fluorescence is observed from the fluorophore. During the polymerization step, the Taq DNA polymerase displaces the Taqman probe by 3-4 nucleotides, and the 5’ nuclease activity of the DNA polymerase

separates the fluorophore from the quencher. The fractional cycle number at which the generated fluorescence passes a fixed threshold above the baseline defines a "Threshold Cycle" (Ct value). A standard curve can be generated from the log of the starting copy number of DNA references against the measured Ct value.

QT-NASBA

Real-time quantitative nucleic acid sequence-based amplification (QT-NASBA) is a sensitive method for detection of sub-microscopic gametocytaemia by measuring gametocyte-specific mRNA. QT-NASBA has been developed for specific detection and quantification of single-stranded RNA in the presence of DNA, regardless of the presence of introns in the target sequence. The NASBA assay developed by AMC-BR targets the specific pfs25 sRNA of the *Plasmodium* gametocyte (specific to late sexual stage of gametocytes). The final output is qualitative because it is not possible to know exactly how much blood is extracted from the filter paper.

APPENDIX B CLINICAL LABORATORIES

Table 10 Outsourced laboratories

Laboratory	Address
Academic Medical Center (AMC) Laboratory for clinical parasitology, L1-247 Department of Medical Microbiology	Meibergdreef 9 1105 AZ Amsterdam The Netherlands

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals

Vaccine Value & Health Science (VVHS)

Protocol Amendment 1

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
Amendment number:	Amendment 1
Amendment date:	21 September 2016
Co-ordinating author:	PPD XPE Pharma & Sciences for GSK Biologicals

1. Risk Management Plan requirement for follow-up extension in EPI-MAL-003 study. EPI-MAL-005 study is conducted in parallel with the EPI-MAL-002 and EPI-MAL-003 studies, and therefore with the extension of EPI-MAL-003, there is need to extend the EPI-MAL-005 study which provides useful data to compare the EPI-MAL-002 and EPI-MAL-003 studies. EPI-MAL-005 study has been extended by four years to nine years.
2. New sites selected for the EPI-MAL-002 and EPI-MAL-003 studies will be included in this study. In addition, World Health Organisation (WHO) proposed the use of RTS,S/AS01$_E$ in moderate to high malaria transmission settings. Due to WHO's proposal, the two sites in Senegal and one site in Tanzania will no longer be participating in this study after Survey 2 because the malaria transmission in these countries is low.
3. The study design was updated to indicate that there is an overlap of EPI-MAL-002 and EPI-MAL-003 with no break.
4. The word "interventional" was removed from the detailed title of the study because this word is no longer in the detailed title of EPI-MAL-002.
5. The tertiary objectives wording has been updated. *P. falciparum* gametocyte NASBA assay was initially expected to be semi-quantitative, but during the method optimisation the assay was more variable than expected. Therefore the test will only be considered as qualitative.
6. The name and address of the laboratory where the NAAT tests will be performed were updated because the activities and projects of laboratory have been transferred to the Academic Medical Centre in Amsterdam.
7. The sample size wording has been updated to reflect the true reason for the limited sample size in children 5-9 years to reach a relative standard error equal to a maximum of 0.35 based on the Pharmacovigilance Risk Assessment Committee (PRAC)'s recommendation.

8. The quantity of blood needed for asexual and sexual (gametocyte) assessment and parasite and gametocyte NAAT assessment has been updated.
9. The criteria for when the rapid diagnostic test will be done has been updated from not only for fever > 37.5°C, but also for subjects with other symptoms/signs of clinical malaria.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Detailed Title	An epidemiology study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in catchment areas of two ~~interventional~~ studies pre- and post RTS,S/AS***01$_E$*** introduction (EPI-MAL-002 and EPI-MAL-003) to assess, in field conditions, vaccine benefit:risk in children in sub-Saharan Africa.
Co-ordinating author	PPD Freelance Science Writer for GSK Biologicals
Contributing authors	• PPD ~~Project Statistician~~ ***Lead Epidemiologist-Malaria*** • PPD ***Oversight Data Manager, Business and Decision Life Sciences for GSK Biologicals*** • PPD Study Data Manager • PPD ***Clinical Laboratory Sciences***-SM, external consultant for GSK Biologicals • PPD ***, Study Delivery Lead***, ***SynteractHCR Benelux NV*** for GSK Biologicals • PPD ***Study Statistician, 4clinics for GSK Biologicals*** • PPD ***Senior Manager, Epidemiologist, ManiAfriCare for GSK Biologicals*** • PPD ~~WHO Fellow, Malaria Team~~ ***Clinical Epidemiologist, Bartech for GSK Biologicals*** • PPD ***Senior Local Delivery Lead*** • PPD ***Global Patent representative***

Protocol *Amendment 1* Sponsor Signatory Approval

Detailed Title	An epidemiology study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in catchment areas of two ~~interventional~~ studies pre- and post RTS,S/AS***01$_E$*** introduction (EPI-MAL-002 and EPI-MAL-003) to assess, in field conditions, vaccine benefit:risk in children in sub-Saharan Africa.
Sponsor signatory	~~Laurence Baril, Head, Global Epidemiology~~ ***François Roman, Clinical & Epidemiology Project Lead, DDW Vaccines***

SYNOPSIS

Detailed Title	An epidemiology study to assess *Plasmodium falciparum* parasite prevalence and malaria control measures in catchment areas of two interventional studies pre- and post RTS,S/AS***01$_E$*** introduction (EPI-MAL-002 and EPI-MAL-003) to assess, in field conditions, vaccine benefit:risk in children in sub-Saharan Africa.
Rationale for the study	Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of ***meningitis,*** protocol defined adverse events of specific interest (***AESI*****,** ***and*** ~~non-communicable and non-traumatic serious~~ ***of other adverse events*** ~~(NC/NT-SAE)~~***.leading to hospitalisation or death, in children***. The first study, EPI-MAL-002, is a surveillance study prior to RTS,S/AS***01$_E$*** authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/ AS0***1$_E$*** safety and will only start when RTS,S/AS0***1$_E$*** is registered and authorised in the country. ***The*** ~~planned age range for licensure of~~ ***World Health Organization's Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC) recommended pilot implementations of RTS,S/AS01$_E$*** ~~candidate~~ ***in children of 5–17 months of age, in parts of 3-5 sub-Saharan African (SSA) countries, administering 3 doses of the vaccine*** ~~is in~~ ***to children*** ~~from 6 weeks~~ ***aged 5-9 months of age*** ~~to less than18 months~~***.in areas of moderate-to-high transmission of malaria with a fourth dose 15-18 months later. The first vaccine introduction is foreseen in 2018.*** The secondary endpoints of these studies ***also*** include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. Up to

seven ***Health and*** Demographic Surveillance System (~~DSS~~ ***H***DSS) (***or equivalent system)*** sites in ~~five~~ countries in Sub-Saharan Africa will participate in these studies, enrolling approximately ~~40~~***3***0 000 children <~~3~~***5*** years of age in ~~each study~~ ***EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003.***

This epidemiology study (EPI-MAL-005) is planned to run in parallel with these two studies, enrolling from the same ~~DSS~~ ***H***DSS ***or (equivalent system)*** populations.

Although not yet ***considered as the*** ~~global~~ ***gold*** standard, NAAT-based (nucleic acid amplification test) techniques can detect infections of lower malaria parasite density and ~~give more accurate~~ quantification of parasitaemia than is achievable by microscopy. Identification of gametocytes by microscopy is inherently challenging, thus their presence is often missed, even by highly trained technicians. Therefore, additional analysis of collected blood samples by NAAT will allow for the detection of lower density parasite infections and will be a more sensitive measure of changes in ***both*** parasite and gametocyte prevalence and density, thereby providing greater insight into potential changes upon vaccine implementation.

Objectives

Secondary

- To estimate longitudinal trends in receipt and timing of the third dose of DTP***/HepB/Hib*** ~~pentavalent~~ and the first dose of measles EPI vaccines, at around 14 weeks and 9 months of age respectively, as appropriate by country.

Tertiary

- To compare asexual and sexual (gametocyte) parasitaemia (qualitative- and ***semi***-quantitative-density) when measured by microscopy or NAAT.

Study design

- Biological samples: A capillary blood sample will be obtained for evaluation of malaria infection by blood slide and NAAT. In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in the last 24 hours ***or other symptoms/signs of clinical malaria,*** a rapid diagnostic test (RDT) will be conducted. If the RDT is positive, treatment will be given according to National guidelines. If a subject for whom no RDT was required is identified as being parasite positive following microscopy, National guidelines should be followed for clinical management of the subject.

- Each study site will be requested annually to provide centre specific information about interventions from the malaria control programme in the study area ~~and, if facilities are available,~~ to provide meteorological data for the study site such as rainfall and temperature. The information will be collected in the form of a questionnaire that will be recorded in a separate database to that for subject-specific data.
- This study will involve up to ~~5~~***9*** annual cross sectional surveys during malaria peak transmission.
- Duration of the study: up to ~~5~~***9*** years

  ~~NOTE: surveys may not be in consecutive years and the total will then be less than 5.~~

  - ***Epoch 006: Survey 6 at Year 6.***
  - ***Epoch 007: Survey 7 at Year 7.***
  - ***Epoch 008: Survey 8 at Year 8.***
  - ***Epoch 009: Survey 9 at Year 9***

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/ Max)	~~No of sites~~	~~Total no of subjects~~	~~Total no of subjects/site~~	Epochs								
					Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 5	Epoch 005 Survey 5	***Epoch 006 Survey 6***	***Epoch 007*** Survey 7	***Epoch 008*** Survey 8	***Epoch 009*** Survey 9
~~Total~~ ***Study site***	6 months / 9 years	~~7~~	~~2100~~	~~3000~~	~~420060~~ 0 subjects	~~4200~~ 600 subjects	~~420060~~ 0 subjects	~~4200~~ 600 subjects	~~420060~~ 0 subjects	***600 subjects***	***600 subjects***	***600 subjects***	***600 subjects***

Discussion of study design

Nine ~~Up to 5~~ annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. Malaria transmission is highly seasonal so surveys will be carried out ~~at the end~~ ***during the course*** of the rainy season ***preferably when rains decrease***, the period of highest malaria transmission.

Number of subjects	Total expected sample size: ~~14000 subjects aged 6 months to <5 years and 7000 subjects aged 5 to <10 years.~~ ***Per site and by survey is a maximum of 400 subjects aged 6 months to <5 years and a maximum of 200 subjects aged 5 to <10 years.***
Endpoints	**Secondary** • Occurrence of uptake and timing of the third dose of DTP***/HepB/Hib*** ~~pentavalent~~ and the first measles EPI vaccines Criteria/definitions: vaccination record of receipt of dose 3 of the DTP***/HepB/Hib*** ~~pentavalent~~ and the first dose measles EPI vaccines.

LIST OF ABBREVIATIONS

AMC	***Academic Medical Center***
eCRF	~~Electronic case report form~~ ***electronic Case Report Form***
GCP	Good ~~clinical practice~~ ***Clinical Practice***
GIS	***Geographic Information System***
HDSS	***Health and*** Demographic surveillance system
HepB	***Hepatitis B***
Hib	***Heamophilus influenza type b***
~~KIT~~	~~Koninklijk Instituut voor de Tropen (Royal Tropical Institute)~~
MPAC	***Malaria Policy Advisory Committee***
PRAC	***Pharmacovigilance Risk Assessment Committee***
SAGE	***Strategic Advisory Group of Experts***
WHO	***World Health Organisation***

GLOSSARY OF TERMS

Catchment area:	In this study, the catchment area is defined as the area of the ***H***DSS (***or equivalent system)*** study site participating in the EPI-MAL-002 and EPI-MAL-003 studies.
DTP/HepB/Hib	***This refers to DTP, or DTP-Hep B (tetravalent) or DTP-HepB-Hib (pentavalent).***
First dose measles	***First measles containing vaccine.***

1 INTRODUCTION

1.1 Background

GSK Biologicals has formulated the candidate pre-erythrocytic *P. falciparum* malaria vaccine, RTS,S/AS***01$_E$***, which is currently under evaluation. The vaccine consists of sequences of the circumsporozoite (CS) protein and hepatitis B surface antigen (HBsAg) adjuvanted with AS01 (liposome formulation with MPL and QS21 immunostimulants).

In the pivotal Phase III study (Malaria-055), efficacy of the candidate malaria vaccine RTS,S/AS01$_E$ against first or only episodes of clinical malaria over a follow-up of 12 months in children aged 5-17 months was 55.8% (p<0.0001), and in coadministration with DTPwHepB/Hib vaccine at 6, 10 and 14 weeks of age was 31.3% (p<0.0001).

1.2 Rationale for the study

Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of ***meningitis,*** protocol defined adverse events of ~~specific~~ ***special*** interest (AESI) and ~~non-communicable and non-traumatic serious~~ ***other adverse events*** ~~(NC/NT-SAE).~~***leading to hospitalisations and death***. The first study, EPI-MAL-002, is a surveillance study prior to RTS,S/AS***01$_E$*** authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/AS***01$_E$*** safety and will only start when RTS,S/AS***01$_E$*** is registered and authorised in the country. The planned age range for licensure of the RTS,S/AS01$_E$ candidate vaccine is in children from 6 weeks of age to less than18 months. The secondary endpoints of these studies include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. Up to seven ***Health and*** Demographic Surveillance System (***H***DSS) (***or equivalent system)*** sites in ~~five~~ ***different*** countries in Sub-Saharan Africa will participate in these studies, enrolling approximately ~~40~~***3***0 000 children <~~3~~***5*** years of age in ~~each study~~ ***EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003.***

This epidemiology study (EPI-MAL-005) is planned to run in parallel with these two studies, enrolling from the same ***H***DSS (***or equivalent system)*** populations.

Although not ***yet considered as*** the ~~global~~ ***gold*** standard, NAAT-based (nucleic acid amplification test) techniques can detect infections of lower malaria parasite density and give more accurate quantification of parasitaemia than is achievable by microscopy. Identification of gametocytes by microscopy is inherently challenging, thus their presence is often missed, even by highly trained technicians. Therefore, additional analysis of collected blood samples by NAAT will allow for the detection of lower density parasite infections and will be a more sensitive measure of changes in ***both*** parasite and gametocyte prevalence and density, thereby providing greater insight into potential changes upon vaccine implementation. This will also provide comparability of data collected in this study with the technique likely to be favoured in clinical trials in the future.

2 OBJECTIVES

2.2 Secondary objectives

- To estimate longitudinal trends in receipt and timing of the third dose of DTP***/HepB/Hib*** ~~pentavalent~~ and the first dose of measles EPI vaccines, at around 14 weeks and 9 months of age respectively, as appropriate by country.

2.3 Tertiary objectives

- To compare asexual and sexual (gametocyte) parasitaemia (qualitative and ***semi***-quantitative-density) ***when measured by microscopy or NAAT*** in the RTS,S/AS01E vaccinated and unvaccinated subjects.
- To compare asexual and sexual (gametocyte) parasitaemia (qualitative- and ***semi***-quantitative-density) when measured by microscopy or NAAT.

3 STUDY DESIGN OVERVIEW

- Biological Samples: A capillary blood sample will be obtained for evaluation of malaria infection by blood slide and NAAT. In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in the last 24 hours ***or other symptoms/signs of clinical malaria,*** a rapid diagnostic test (RDT) will be conducted. If the RDT is positive, treatment will be given according to National guidelines. If a subject for whom no RDT was required is identified as being parasite positive following microscopy, National guidelines should be followed for clinical management of the subject.

Using ***Geographic Information System*** (GIS) to determine geographical variability in MTI locally: Study areas will be mapped by villages using grid referencing.

- This study will involve up to ~~5~~***9*** annual cross sectional surveys during malaria peak transmission.
- Duration of the study: up to ~~5~~***9*** years

  ~~NOTE: surveys may not be in consecutive years and the total will then be less than 5.~~

  - ***Epoch 006: Survey 6 at Year 6.***
  - ***Epoch 007: Survey 7 at Year 7.***
  - ***Epoch 008: Survey 8 at Year 8.***
  - ***Epoch 009: Survey 9 at Year 9***

Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/Max)	~~No of sites~~	~~Total no of subjects~~	~~Total no of subjects/site~~	Epochs								
					Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 4	Epoch 005 Survey 5	***Epoch 006 Survey 6***	***Epoch 007*** Survey 7	***Epoch 008*** Survey 8	***Epoch 009*** Survey 9
~~Total~~ ***Study site***	6 months / 9 years	~~7~~	~~2100~~	~~3000~~	~~4200~~600 subjects	~~4200~~ 600 subjects	~~4200~~600 subjects	~~4200~~ 600 subjects	~~4200~~600 subjects	***600 subjects***	***600 subjects***	***600 subjects***	***600 subjects***

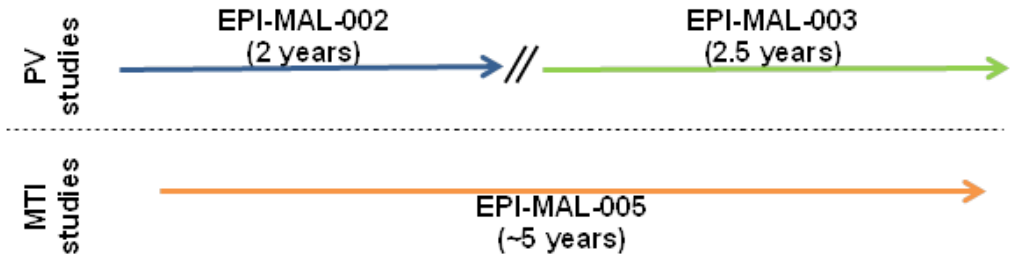
3.1 Discussion of study design

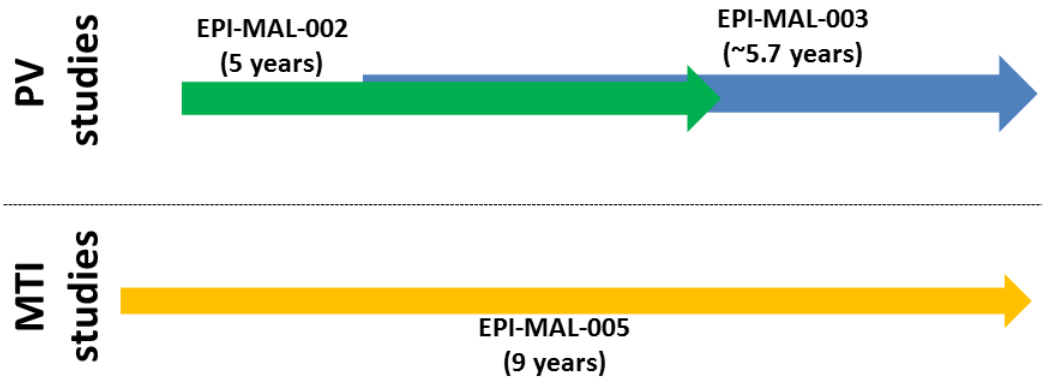
~~Up to 5~~ ***Nine*** annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. Malaria transmission is highly seasonal so surveys will be carried out ~~at~~ ***during the*** ~~end~~ ***course of the rainy season preferably when rains decrease***, the period of highest malaria transmission.

4 STUDY POPULATION

4.1 Number of subjects/ centres

Subjects 6 months to <10 years of age will be enrolled in ***H***DSS (***or equivalent system)*** catchment areas at the sites participating in the EPI-MAL-002 and EPI-MAL-003 studies of the candidate malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa. Up to ~~7~~ ***seven*** ***H***DSS (***or equivalent system)*** sites ~~will~~ ***are planned to*** participate in this study (see Section 4.1.1). Subjects enrolled in EPI-MAL-002 and EPI-MAL-003 are eligible for participation in this study.





Each study site will enroll approximately 600 subjects per survey. ***These subjects will be selected at random from the HDSS (or equivalent system) population listings prepared at each site. The selection process will be repeated every year meaning that the subjects will be different in each cross-sectional survey except if they are re-selected in a subsequent survey by chance***. ***The*** population ***listings*** generated from the demographic surveillance will allow for sampling of the required subjects according to stratification by age group as follows (all subject numbers are approximately plus or minus 5 children):

Therefore, the total expected sample size ~~over 5 surveys~~ ***per site and by survey is a maximum of*** ~~14000~~ ***400 subjects aged 6 months to <5 years and a maximum of*** ~~7000~~ ***200 subjects aged 5 to <10 years***, ~~calculated as follows:~~

- ~~Subjects aged 6 months to <5 years: max 5 study years x 7 sites x 400 subjects = max 14000 subjects~~
- ~~Subjects aged 5 to <10 years: max 5 study years x 7 sites x 200 subjects = max 7000 subjects.~~

4.1.1 Recruitment of study centres/ investigators

All ***H***DSS ***(or equivalent system)*** study sites participating in EPI-MAL-002 and EPI-MAL-003 will be approached with regard to participation in this epidemiological study. At the time of ***original*** protocol finalisation, these comprised ~~centres~~ ***sites*** in Burkina Faso, Ghana, Senegal, Tanzania, and Kenya ~~(APPENDIX A).~~

The study sites will be revised after survey 2. Following the World Health Organization (WHO)'s Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC) recommendations of pilot implementations of RTS,S/AS01$_E$ in 3-5 distinct settings in sub-Saharan Africa (SSA) restricted to moderate-to-high transmission of malaria, other settings in SSA with moderate-to-high transmission of malaria might be added to the already defined EPI-MAL-002 and EPI-MAL-003 study sites. These new sites will also be added to the EPI-MAL-005 study.

The study sites in Senegal and Tanzania with low malaria transmission will no longer participate in the study after Survey 2.

4.1.2 Sampling methods

All participating centres will have a ***H***DSS ***(or equivalent system)*** in place. In general, any variation in sampling procedure should follow as closely as possible the Guidelines of the Roll Back Malaria Monitoring and Evaluation Reference Group [2005]. The following steps serve as an example of a sampling method.

1. Generate a list from the ***H***DSS ***(or equivalent system)*** database of all children aged 6 months to <10 years.

Each study site will enrol approximately 600 subjects per survey. To allow for 10% non-response 660 subjects should therefore be randomly selected from the ***H***DSS ***(or equivalent system)*** database distributed over all age categories as described in Section 4.1.

5.4 Outline of study procedures

Table 2 details study procedures to be conducted during the study.

Table 2 List of study procedures to be conducted at each survey visit

Epoch	**Epoch 001**	**Epoch 002**	**Epoch 003**	**Epoch 004**	**Epoch 005**	***Epoch 006***	***Epoch 007***	***Epoch 008***	***Epoch 009***
Study Group[1]	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	***Survey 6***	***Survey 7***	***Survey 8***	***Survey 9***
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	***Year 6***	***Year 7***	***Year 8***	***Year 9***
Informed consent	●	●	●	●	●	●	●	●	●
Check inclusion/exclusion criteria	●	●	●	●	●	●	●	●	●
Record demographic details	●	●	●	●	●	●	●	●	●
Record subject's vaccination status[2]	●	●	●	●	●	●	●	●	●
Record any anti-malarial medication or other medication received within the previous 14 days	●	●	●	●	●	●	●	●	●
Record relevant medical history (hospitalisation, reported fever, and care seeking behaviour)	●	●	●	●	●	●	●	●	●
Record axillary body temperature	●	●	●	●	●	●	●	●	●
Record malaria control measures used in the household	●	●	●	●	●	●	●	●	●
Record malaria prevention and risk factors	●	●	●	●	●	●	●	●	●
Take capillary blood sample ~~(up to 500 µL)~~[3]	●	●	●	●	●	●	●	●	●
Assessment of RDT[4]	●	●	●	●	●	●	●	●	●
Study conclusion	●	●	●	●	●	●	●	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.

1. ***The new sites will join the study after survey 3, depending on when they are included in EPI-MAL 002/ 003. The total surveys at these new sites will therefore be less than 9***~~. Surveys may not be in consecutive years and the total will then be less than 5~~
2. RTS,S/AS01 and 3rd dose DTP/***HepB/Hib*** and first dose measles EPI vaccinations, only
3. Capillary blood sample for determination of parasite prevalence (blood slides and NAAT by filter paper)
4. ~~Only~~ In the event of measured fever at time of visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours ***or other symptoms/signs of clinical malaria***, a rapid diagnostic test (RDT) will be conducted using capillary blood sample taken for blood slide preparation.

Table 3 List of study procedures to be conducted during survey

Epoch	Epoch 001	Epoch 002	Epoch 003	Epoch 004	Epoch 005	*Epoch 006*	*Epoch 007*	*Epoch 008*	*Epoch 009*
Study Group[1]	Survey 1	Survey 2	Survey 3	Survey 4	Survey 5	*Survey 6*	*Survey 7*	*Survey 8*	*Survey 9*
Timepoint	Year 1	Year 2	Year 3	Year 4	Year 5	*Year 6*	*Year 7*	*Year 8*	*Year 9*
Site specific information collection - Meteorological data ~~(if available)~~ [1]	●	●	●	●	●	●	●	●	●
Site specific information collection – Malaria Control Programme	●	●	●	●	●	●	●	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.
~~Surveys may not be in consecutive years and the total will then be less than 5.~~ Availability of meteorological data will be based on the site having the weather station. All sites received weather stations either post Survey 1 or 2.

5.5.4 Check and record medications/vaccinations

Record RTS,S/AS01*E* (all doses) and other vaccinations (i.e. 3rd dose DTP***/HepB/Hib*** ~~pentavalent~~ and first dose measles EPI vaccinations) administered, including dates. The vaccination status will be recorded in the eCRF.

5.5.7 Collect data on malaria control interventions

Record the following malaria control measures in the subject's eCRF:

- Residual spraying, mosquito net usage, seasonal malaria chemoprevention (SMC), intermittent ~~preventative~~ ***preventive*** treatment in infants (IPTi), and ~~ACT~~ ***all anti-malaria*** therapy received within the last 14 days.

5.5.9 Capillary blood sampling

A capillary blood sample ~~(up to 500µL),~~ collected by finger/heel prick, will be taken during the study visit for the assessment of parasitaemia (see Section 5.6.2). The following biological samples will be taken at each survey visit:

- Blood for asexual and sexual (gametocyte) microscopy (2 slides ~~[up to 4 slides might be prepared according to study site standard operating procedures (SOPs)].~~ approximately 2 drops on each microscope slide).

In the event of measured fever at the time of the visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours ***or other symptoms/signs of clinical malaria***, a RDT will also be conducted using the capillary blood sample.

5.5.10 Site specific information collection

All sites received a weather station either post Survey 1 or Survey 2.

5.6 Biological sample handling and analysis

- It is also possible that future findings may make it desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all subjects in countries where this is allowed ***when signing the Informed Consent Form*** will be invited to give another specific consent to allow GSK or a contracted partner ***to*** use the samples for future research including development of tests and their quality assurance.

5.6.2 Biological samples

Table 4 Biological samples

Sample type	Quantity	~~Sample volume (max)~~	Timepoint
Whole blood	Approximately 1 drop (RDT assessment)*	~~0.06~~	Survey Visit
Whole blood	Approximately 4 drops; ***1 drop for the thin smear and up to 3 drops for the thick smear*** ~~up to 2 drops on each microscope slide~~ (merozoite and gametocyte microscopy assessment)	~~0.24 mL~~	Survey Visit
Whole blood	~~Approximately~~ 2 ***to 3*** drops; filter paper collection parasite and gametocyte NAAT assessment)	~~0.12 mL~~	Survey Visit

RDT = Rapid Diagnostic Test
NAAT = Nucleic Acid Amplification Test
*~~Only in~~ In the event of measured fever at time of visit (axillary temperature ≥37.5°C) or reported fever in last 24 hours ***or other symptoms/signs of clinical malaria.***

5.6.3 Laboratory assays

Please refer to ~~APPENDIX B~~ ***APPENDIX A*** for a detailed description of the assays performed in the study. Please refer to ~~APPENDIX C~~ ***APPENDIX B*** for the address of the clinical laboratories used for sample analysis.

In the event of fever measured at time of visit (axillary temperature ≥37.5°C) or reported fever in last 24 hours ***or other symptoms/signs of clinical malaria***, a RDT will be conducted. The clinical management of subjects presenting with malaria as identified by RDT or microscopy will follow National guidelines. Subjects will receive appropriate care according to standard clinical practice.

All blood slides with thick and thin smears will be made in duplicate. ~~In the study sites where study site SOPs require the preparation of separate thin and thick smears, up to 4 blood slides might be prepared.~~ One slide with thick and thin smears, ~~or two slides with separate thick and thin smears,~~ will be sent for storage to a GSK dedicated laboratory facility and the remaining slide~~(s)~~ will be stored at the study site. For each slide, parasitaemia will be determined independently by two readers and in the case of non-concordance an additional read by a third independent reader will be carried out. Since there will be no full blood count performed in the study subjects the used methodology for the determination of *P. falciparum* asexual parasite density may be based on an assumed white cell count of 8000/µL. Please refer to ~~APPENDIX B~~ ***APPENDIX A*** for a description of other methodologies for the determination ***of*** *P. falciparum* asexual parasite density.

Table 5 Molecular Biology (NAAT tests)

System	Component	Method	Unit	Laboratory*
3667	18S parasite DNA	QT-PCR *Plasmodium falciparum*	Semi-quantitative results (e.g. H, M, L)	~~KIT~~ ***AMC***
3668	Pfs25 gametocyte mRNA	QT-NASBA gametocyte	~~Semi-quantitative results (e.g. H, M, L)~~ ***Qualitative***	~~KIT~~ ***AMC***
3669	GAPDH parasite DNA	PCR GAPDH	Positive/Negative	~~KIT~~ ***AMC***
3670	β2 microglobuline ~~gametocyte~~ mRNA	RT-PCR β2 microglobuline	Positive/Negative	~~KIT~~ **AMC**

*Refer to APPENDIX B ~~APPENDIX C~~ for the laboratory addresses
~~KIT: Koninklijk Instituut voor de Tropen (Royal Tropical Institute)~~
AMC: Academic Medical Center

7.1.2 Secondary endpoints

- Occurrence of uptake and timing of the third dose of DTP***/HepB/Hib*** ~~pentavalent~~ and the first measles EPI vaccines

  Criteria/definitions: vaccination record of receipt of dose 3 of the DTP***/HepB/Hib*** ~~pentavalent~~ and the first dose ***of the*** measles EPI vaccines.

7.2.3 Children aged 5 to < 10 years

Since the precision of 0.25 was adequate and determined for the WHO (children aged 6 months to <5 years) and JTEG (children aged 2 to <10 years) definitions, the sample size for the subgroup of children 5-9 years was fixed at 200 which gives a relative standard error equal to a maximum of 0.35. In addition, it is anticipated that children in this older age group are more likely to be at school and it may take longer to recruit this age group with subsequent logistics and financial implications.

7.7.1.1 Overall

First the trends in parasite prevalence between each cross-sectional malariometric surveys will be tested using the Cochran-Armitage trend test [Agresti, 1990]. This hypothesis test will be performed on the parasite prevalence ***computed on the independent samples of subjects in each survey,*** within each site separately.

7.7.1.2.1 Annual fluctuations: vaccine ineligible subgroup

Once the cross-surveys are collected, the independent samples of subjects in each survey will be ***tested and*** assessed using a Chi-square statistic to test the significance of the differences among the parasite prevalences in each survey; meaning annual fluctuations.

7.7.2 Other prevalences and proportions

Prevalence ~~estimate~~ for each segment will be estimated using the following criteria for level of endemicity: ‘high’ ≥ 60%, ‘moderate’ 20%-59%, and ‘low’ <20% relating to the proportion of infected children in each segment [Snow 1997; Omumbo 1998].

The proportion of subjects having received the third dose of DTP/***HepB/Hib*** ~~pentavalent~~ and the first dose of measles EPI vaccine will be estimated as the ratio of the subjects having received the third dose of DTP/***HepB/Hib*** ~~pentavalent~~ and the first dose of

measles EPI vaccine divided by the number of subjects who should have received the third dose of DTP/pentavalent and the first dose of measles EPI (i.e. 14 weeks and 9 months of age, respectively). Moreover, among the subjects having received the third dose of DTP/***HepB/Hib*** ~~pentavalent~~ and the first dose of measles EPI vaccine, the proportions of subjects having received them on time, in advance and with delay will be estimated.

7.8 Analysis of tertiary objectives

The comparisons of asexual and sexual (gametocyte) parasitaemia measured by microscopy and NAAT will be done using ***Cohen's Kappa coefficient*** ~~Fisher's exact test~~. The hypothesis test will be done per centre, and overall. ~~No correction for multiple testing will be done.~~

7.10.1 Sequence of analyses

Statistical reports will be written after each epoch. A clinical report will be written ***after*** ~~each epoch by GSK~~ ***Surveys 2 and 5 and an integrated report will be written at the end of the study.***

~~APPENDIX A STUDY SITES~~

~~PI Last Name~~	~~PI First Name~~	~~Country- Study Site~~	~~Location~~
~~Owusu-Agyei~~	~~Seth~~	~~GHANA - KINTAMPO~~	~~Kintampo Health Research Centre~~ Kintampo GHANA
~~Otieno~~	~~Walter~~	~~KENYA - KOMBEWA~~	~~Walter Reed Project - Kombewa Clinical Research Centre~~ Opposite Kisumu West District Hospital Kisumu Bondo Road Kisumu KENYA
~~Lusingu~~	~~John~~	~~TANZANIA - KOROGWE~~	~~Joint Malaria Programme (JMP)~~ Korogwe District Hospital, New Laboratory Building, Korogwe, Tanga TANZANIA
~~Gaye~~	~~Omar~~	~~SENEGAL – KEUR SOCE~~	~~Centre de Recherche de Keur Socé~~ ~~Departement de Parasitologie~~ Faculté de Médecine Université Cheikh Anta Diop, Dakar SENEGAL
~~Sokhna~~	~~Cheikh S~~	~~SENEGAL - NIAKHAR~~	~~UMR 198 URMITE~~ ~~Unite de Recherche sur les Maladies Infectieuses et Tropicales Emergentes~~ Campus commun IRD-UCAD de Hann BP 1386 CP 18524 Dakar SENEGAL
~~Bienvenu Sirima~~	~~Sodiomon~~	~~BURKINA FASO - SAPONE~~	~~Centre National de recherche et de Formation sur le paludisme (CNRFP)~~ 01 BP 2208 Ouagadougou 01 BURKINA FASO
~~Sie~~	~~Ali~~	~~BURKINA FASO - NOUNA~~	~~Centre de Recherche en Santé de Nouna,~~ ~~PO BOX 02 Nouna~~ ~~BURKINA FASO~~

APPENDIX A

DETERMINATION OF PARASITAEMIA BY NAAT

Q-PCR

The real time Q-PCR assay developed by ~~KIT~~ ***AMC***-BR targets the 18S gene of the *Plasmodium falciparum.*

QT-NASBA

The NASBA assay developed by ~~KIT~~ **AMC**-BR targets the specific pfs25 sRNA of the *Plasmodium* gametocyte (specific to late sexual stage of gametocytes). The final output is ***qualitative*** ~~semi-quantitative (e.g. High, Medium, Low)~~ because it is not possible to know exactly how much blood is extracted from the filter paper.

APPENDIX ~~C~~ ***B*** CLINICAL LABORATORIES

Table 10 Outsourced laboratories

Laboratory	Address
Koninklijk Instituut voor de Tropen (KIT) (Royal Tropical Institute) ***Academic Medical Center (AMC)*** Laboratory for clinical parasitology, L1-247 Department of Medical Microbiology	Meibergdreef 39 ***9*** 1105 AZ Amsterdam The Netherlands

GlaxoSmithKline Biologicals SA

Vaccines R & D

Protocol Amendment 2

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
Amendment number:	Amendment 2
Amendment date:	14 September 2018
Co-ordinating author:	PPD Freelance Science Writer for GSK Biologicals

Rationale/background for changes:

A delay in the implementation of the RTS,S/AS01$_E$ vaccine in the 3 countries participating in the World Health Organisation (WHO)-led Malaria Vaccine Implementation Programme (MVIP) has resulted in a delay to the start of EPI-MAL-003. As a consequence, EPI-MAL-005, being an ancillary study to EPI-MAL-003, will need to be extended in order to mirror the EPI-MAL-003 timeline. The need for an additional survey is the trigger for the second amendment of this protocol. Additional revisions to the protocol are listed below.

1. The current protocol – protocol amendment 1 - currently details 9 annual surveys with study duration of up to 9 years. The study will include at least 1 further annual survey, lasting for at least 10 years in total. Thus, the longest participating sites will perform the study for at least 10 years meaning 10 surveys, whilst some sites joining later will not perform as many surveys. Protocol text has been revised accordingly.
2. Country and study site participation have changed during the course of the study following WHO recommendations for pilot implementation of RTS,S/AS01$_E$ in settings with moderate-to-high transmission of malaria and WHO Regional Office for Africa selection for the initial introduction of RTS,S/AS01$_E$ in 3 countries (Ghana, Kenya and Malawi) through the MVIP. EPI-MAL-003 study site selection will be performed following Ministries of Health pre-selection and according to WHO guidance in the framework of the MVIP, to include 4 sites in each of the 3 countries selected for the MVIP. Being an ancillary study to EPI-MAL-002 and EPI-MAL-003 studies, EPI-MAL-005 is/will be conducted in study sites conducting EPI-MAL-002 and/or EPI-MAL-003. Clarification on the countries and sites participating in the study has been provided.
3. In response to a Corrective and Preventive Action following a Critical Quality Attributes audit conducted in Kintampo, Ghana, reference to study conduct in accordance with ‘Good Pharmacoepidemiology Practices (GPP) guidelines’ has been added to Section 5.1 ‘Regulatory and ethical considerations, including the informed consent process’ to align with STD-POL-GSKF-408, and additionally protocols EPI-MAL-002 and EPI-MAL-003.

4.	Vaccine eligible and ineligible subgroups were defined based on assumptions on the duration of follow-up of the EPI-MAL-005 study and date of vaccine implementation. This amendment primarily documents the extension of the study for at least 1 further survey. Furthermore, the date of implementation of the vaccine has yet to be confirmed. It is therefore not possible to apply these assumptions and the applicable text in Section 7.7.1.2 'By vaccine eligible/ineligible subgroup: Statistical analyses for vaccine association adjustments and association between vaccine and transmission' has been revised.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Contributing authors

- ……
- PPD ***Clinical Manager Safety Physician***
- PPD ***Regional Clinical Operations Head~~,~~*** ~~Senior Manager, Global Study Management~~
- PPD Clinical Laboratory Sciences-SM Business and Decision Life Sciences ~~/ external consultant~~ for GSK Biologicals
- ……..
- PPD ***Local Delivery Lead***
- PPD external consultant for GSK Biologicals
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- PPD ***Lead Epidemiologist-Malaria, Aixial for GSK Biologicals***
- ……..
- PPD ***Senior Lead Epidemiologist-Malaria***
- PPD Study Data Manager, ***TCS for GSK Biologicals***
- PPD ***Clinical & Epidemiology Project Lead, DDW Vaccines***
- PPD ~~Senior Local Delivery Lead~~ ***Country Clinical Operations Head, Africa and Middle East***
- ……
- PPD Oversight Data Manager~~, Business and Decision Life Sciences for GSK Biologicals~~
- PPD ~~and~~ PPD Study Data Manager
- PPD ***Study Statistician***

Protocol Amendment 2 Investigator Agreement

I agree:

- ……..
- To assume responsibility for the proper conduct of the study at ~~this~~ ***the*** site***(s)***.

SYNOPSIS

Rationale for the study	Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of meningitis, protocol defined adverse events of ~~specific~~ ***specific*** interest (AESI), and of other adverse events leading to hospitalisation or death, in children. The first study, EPI-MAL-002, is a ***baseline*** surveillance study prior to RTS,S/AS01$_E$ authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/AS01$_E$ safety***, as well as vaccine effectiveness and impact,*** and will only start when RTS,S/AS01$_E$ is ~~registered and~~ authorised ***and implemented*** in the country. The World Health Organi~~sz~~ation's ***(WHO's)*** Strategic Advisory Group of Experts (SAGE) on Immunization and the Malaria Policy Advisory Committee (MPAC) recommended pilot implementations of RTS,S/AS01$_E$ in children of 5–17 months of age, in parts of 3-5 sub-Saharan African ~~(SSA)~~ countries, administering 3 doses of the vaccine to children aged 5-9 months of age in areas of moderate-to-high transmission of malaria with a fourth dose 15-18 months later. The first vaccine introduction is foreseen in 2018. The secondary endpoints of these studies also include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. ~~Up to seven~~ Health and Demographic Surveillance System (HDSS) (or equivalent system) sites***[1]*** in countries in ~~S~~***s***ub-Saharan Africa will participate in these studies, enrolling approximately 30 000 children <5 years of age in EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003. This epidemiology study (EPI-MAL-005) is planned to run in parallel with these two studies, enrolling from the same HDSS ***(***or ~~(~~equivalent system) populations. The primary objectives of

this study are to produce longitudinal estimates of parasite prevalence in humans, and record malaria control measure usage in areas where EPI-MAL-002 and EPI-MAL-003 studies will take place.……..

Outside of the controlled setting of a clinical trial, there is the risk that usage of malaria control interventions such as ***indoor*** residual spraying and bednets may change following vaccine introduction. ……

[1] Throughout the document, the terms of site, cluster and centre are used interchangeably, having the same meaning.

Study design

- Type of design: A multi-centric, epidemiology longitudinal cross-sectional study at centres in ~~S~~***s***ub-Saharan Africa that are participating in GSK's EPI-MAL-002 and EPI-MAL-003 studies.
- …….
- Using ***Geographic Information System (***GIS***)*** to determine geographical variability in MTI locally: Study areas will be mapped by villages using grid referencing. Subjects will be attributed to their village, however, to avoid PII (personally identifiable information); small villages will be grouped when the number of participants is less than 10, ~~such~~ ***so*** that it is not possible to identify one subject from one village. ……..
- ……
- This study will involve up to ~~9~~ ***10*** annual cross sectional surveys during malaria peak transmission ***with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies***.
- Duration of the study: up to ~~9~~ ***10*** years ***with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies***:
  - ……..
  - ***Epoch 010: Survey 10 at Year 10***

Synopsis Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/Max)	Epochs									
		Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 4	Epoch 005 Survey 5	Epoch 006 Survey 6	Epoch 007 Survey 7	Epoch 008 Survey 8	Epoch 009 Survey 9	***Epoch 010 Survey 10***
Study site	6 months / 9 years	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	***600 subjects***

Discussion of study design	……. ~~Nine~~ ***At least 10*** annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. ……
Endpoints	**Primary** • Occurrence of *P. falciparum* parasitaemia (using microscopy) Criteria/definitions: infection with *P. falciparum* determined using a blood smear slide and determined using microscopy. • Occurrence of malaria control interventions Criteria/definitions: malaria control interventions are mosquito net usage ***(including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN]), indoor*** residual spraying ***(IRS)***, seasonal malaria chemoprevention (SMC), intermittent preventative treatment in infants (IPTi), and ACT therapy received within the last 14 days.

LIST OF ABBREVIATIONS

AESI	Adverse events of ~~specific~~ ***special*** interest
GPP	***Good Pharmacoepidemiology Practice***
LLIN	***Long lasting insecticidal nets***
MVIP	***Malaria Vaccine Implementation Programme***

GLOSSARY OF TERMS

Malaria control interventions:	Any intervention or control measure targeted at reducing malaria transmission or preventing malaria disease [World Health Organisation, 2013]. This includes ITN, ***LLIN,*** IRS, SMC, IPTi, ***IPTp*** and ACTs.

1.2 Rationale for the study

Following the pivotal Phase III study of the candidate malaria vaccine RTS,S/AS01$_E$ (Malaria-055), two consecutive vaccine safety monitoring studies (EPI-MAL-002 and EPI-MAL-003) will be conducted to monitor incidence rates of meningitis, protocol defined adverse events of ~~special~~ ***specific*** interest (AESI), and ***of*** other adverse events leading to hospitalisation~~s and~~ ***or*** death***, in children***. The first study, EPI-MAL-002, is a ***baseline*** surveillance study prior to RTS,S/AS01$_E$ authorisation in the country; the second study, EPI-MAL-003, will more specifically monitor RTS,S/AS01$_E$ safety***, as well as vaccine effectiveness and impact,*** and will only start when RTS,S/AS01$_E$ is ~~registered and~~ authorised ***and implemented*** in the country. ***The World Health Organisation's (WHO's) Strategic Advisory Group of Experts (SAGE) on immunization and the Malaria Policy Advisory Committee (MPAC) recommended pilot implementations of RTS,S/AS01$_E$ in children of 5–17 months of age, in parts of 3-5 sub-Saharan African ~~(SSA)~~ countries, administering 3 doses of the vaccine to children aged 5-9 months of age in areas of moderate-to-high transmission of malaria with a fourth dose 15-18 months later. The first vaccine introduction is foreseen in 2018.*** ~~The planned age range for licensure of the RTS,S/AS01$_E$ candidate vaccine is in children from 6 weeks of age to less than18 months.~~ The secondary endpoints of these studies ***also*** include monitoring the incidence of malaria disease as diagnosed during out-patient visits or hospitalisation. ~~Up to seven~~ Health and Demographic Surveillance System (HDSS) (or equivalent system) sites[2] in ~~different~~ countries in ~~S~~***s***ub-Saharan Africa will participate in these studies, enrolling approximately 30 000 children <5 years of age in EPI-MAL-002 and approximately 45 000 children <5 years in EPI-MAL-003.

……..

Outside of the controlled setting of a clinical trial, there is the risk that usage of malaria control interventions such as ***indoor*** residual spraying and bednets may change following vaccine introduction. …….

[2] Throughout the document, the terms of site, cluster and centre are used interchangeably, having the same meaning.

3. STUDY DESIGN OVERVIEW

……

- Type of design: A multi-centric, epidemiology longitudinal cross-sectional study at centres in ~~S~~***s***ub-Saharan Africa that are participating in GSK's EPI-MAL-002 and EPI-MAL-003 studies.
- …….
- Using Geographic Information System (GIS) to determine geographical variability in MTI locally: Study areas will be mapped by villages using grid referencing. Subjects will be attributed to their village, however, to avoid PII (personally identifiable information), small villages will be grouped when the number of participants is less than 10, ~~such~~ ***so*** that it is not possible to identify one subject from one village. ….

- ……

- This study will involve up to ~~9~~ ***10*** annual cross sectional surveys during malaria peak transmission ***with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies***.

- Duration of the study: up to ~~9~~ ***10*** years ***with possible further extension, dependent on the duration of the EPI-MAL-002 and EPI-MAL-003 studies:***

  – ……

  – ***Epoch 010: Survey 10 at Year 10***

Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/Max)	Epochs									
		Epoch 001 Survey 1	Epoch 002 Survey 2	Epoch 003 Survey 3	Epoch 004 Survey 4	Epoch 005 Survey 5	Epoch 006 Survey 6	Epoch 007 Survey 7	Epoch 008 Survey 8	Epoch 009 Survey 9	***Epoch 010 Survey 10***
Study site	6 months / 9 years	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	600 subjects	***600 subjects***

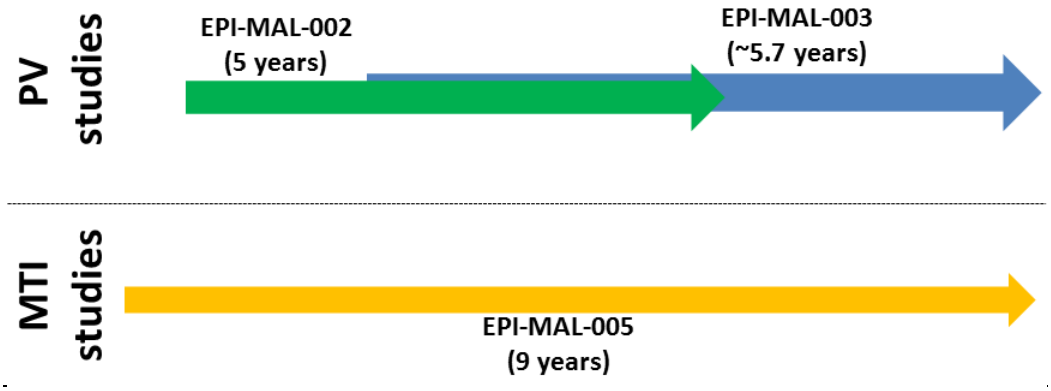
3.1. Discussion of study design

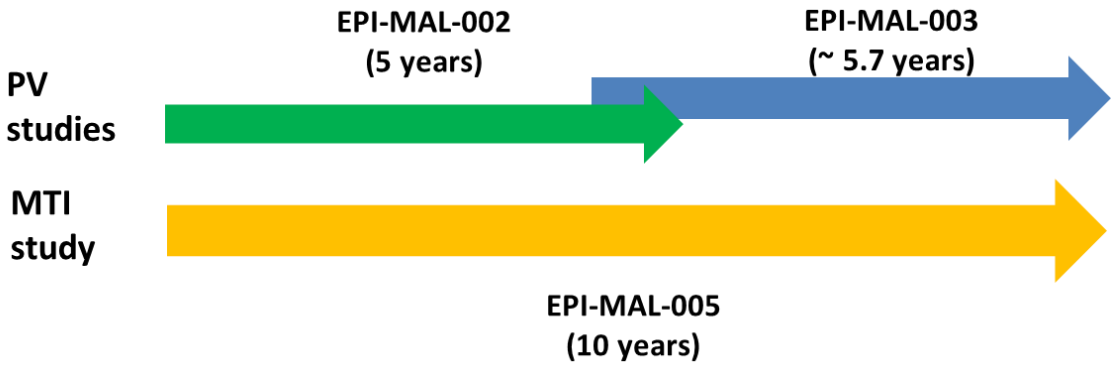
……..

~~Nine~~ ***At least 10*** annual cross sectional surveys at peak transmission will provide point estimates of parasite prevalence and subsequently a longitudinal assessment of the level of endemicity in each area covered by EPI-MAL-002 and EPI-MAL-003. …….

4.1. Number of subjects/ centres

Subjects 6 months to <10 years of age ~~will be enrolled~~ ***living*** in HDSS (or equivalent system) catchment areas ~~at~~ ***of*** the sites participating in the EPI-MAL-002 and EPI-MAL-003 studies ~~of the candidate malaria vaccine RTS,S/AS01$_E$ in sub-Saharan Africa~~ ***are eligible for enrolment***~~. Up to seven HDSS (or equivalent system) sites are planned to participate in this study~~ (see Section 4.1.1). Subjects ***already*** enrolled in EPI-MAL-002 and EPI-MAL-003 are eligible for participation in this study.





4.1.1 Recruitment of study centres / investigators

~~All HDSS (or equivalent system) study sites participating in EPI-MAL-002 and EPI-MAL-003 will be approached with regard to participation in this epidemiological study.~~ ***The ancillary EPI-MAL-005 study will be conducted in sites defined in the EPI-MAL-002 and EPI-MAL-003 protocols.*** At the time of original protocol finalisation, ***identified study sites were located*** ~~these comprised sites~~ in Burkina Faso, Ghana, ***Kenya,*** Senegal, ***and*** Tanzania~~, and Kenya~~.

~~The study sites will be revised after survey 2.~~ Following the ~~World Health Organization (~~WHO~~)~~'s ~~Strategic Advisory Group of Experts (~~SAGE~~)~~ on immunization and the ~~Malaria Policy Advisory Committee (~~MPAC~~)~~ recommendations of pilot implementations of RTS,S/AS01$_E$ in 3-5 distinct settings in sub-Saharan Africa ~~(SSA)~~ restricted to moderate-to-high transmission of malaria, ~~other settings in SSA with moderate-to-high transmission of malaria might be added to the already defined EPI-MAL-002 and EPI-MAL-003 study sites. These new sites will also be added to the EPI-MAL-005 study.~~ ***site selection in some of the initially chosen EPI-MAL-002, EPI-MAL-003 and EPI-MAL-005 study sites located in low endemicity settings (i.e. in Senegal and Tanzania) had to be terminated. In April 2017, the WHO Regional Office for Africa announced that the RTS,S/AS01$_E$ vaccine will be first introduced in 3 countries (Ghana, Kenya and Malawi) through the Malaria Vaccine Implementation Programme (MVIP). Selection of the clusters that are/will participate in GSK's baseline, Phase IV and ancillary studies (i.e. EPI-MAL-002, EPI-MAL-003 and EPI-MAL-005, respectively), being fully embedded in the MVIP, depends on the cluster identification process led by the Ministries of Health according to WHO guidance. They have been, or will be, selected as follows:***

- ***Sites have been, or will be, selected from the 3 countries where the RTS,S/AS01$_E$ vaccine will be implemented (Ghana, Kenya and Malawi). Burkina Faso sites that started EPI-MAL-005 will early terminate the conduct of the study following Survey 4; data from these sites will be presented in the interim report planned following Survey 5.***
- ***As currently planned in the MVIP and according to WHO guidance, 4 study sites (corresponding to 4 clusters of the MVIP) in each of the 3 countries selected for the RTS,S/AS01$_E$ pilot implementation programme (12 study sites/clusters in total) are planned to be part of EPI-MAL-005.***

- ***Of note, all study sites are submitted to a comprehensive scientific and operational study site assessment conducted by GSK, which will determine study feasibility in those sites.***

~~The study sites in Senegal and Tanzania with low malaria transmission will no longer participate in the study after Survey 2.~~

In summary, selection of sites will be performed in EPI-MAL-002 and EPI-MAL-003 studies following Ministries of Health pre-selection and according to WHO guidance, to include 4 sites in each of the 3 countries selected for the MVIP. EPI-MAL-005 is/will be conducted in study sites conducting EPI-MAL-002 and/or EPI-MAL-003.

5.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP)***, Guidelines for Good Pharmacoepidemiology Practices (GPP) [ISPE, 2015]***, ~~or~~ other applicable guidelines, all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

5.4. Outline of study procedures

Table 2 List of study procedures to be conducted at each survey visit

Epoch	**Epoch 001**	**Epoch 002**	**Epoch 003**	**Epoch 004**	**Epoch 005**	**Epoch 006**	**Epoch 007**	**Epoch 008**	**Epoch 009**	***Epoch 010***
Study Group[1]	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Survey 6**	**Survey 7**	**Survey 8**	**Survey 9**	***Survey 10***
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	***Year 10***
Informed consent	●	●	●	●	●	●	●	●	●	●
Check inclusion/exclusion criteria	●	●	●	●	●	●	●	●	●	●
Record demographic details	●	●	●	●	●	●	●	●	●	●
Record subject's vaccination status[2]	●	●	●	●	●	●	●	●	●	●
Record any anti-malarial medication or other medication received within the previous 14 days	●	●	●	●	●	●	●	●	●	●
Record relevant medical history (hospitalisation, reported fever, and care seeking behaviour)	●	●	●	●	●	●	●	●	●	●
Record axillary body temperature	●	●	●	●	●	●	●	●	●	●
Record malaria control measures used in the household	●	●	●	●	●	●	●	●	●	●
Record malaria prevention and risk factors	●	●	●	●	●	●	●	●	●	●
Take capillary blood sample[3]	●	●	●	●	●	●	●	●	●	●

Epoch	**Epoch 001**	**Epoch 002**	**Epoch 003**	**Epoch 004**	**Epoch 005**	**Epoch 006**	**Epoch 007**	**Epoch 008**	**Epoch 009**	***Epoch 010***
Study Group[1]	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Survey 6**	**Survey 7**	**Survey 8**	**Survey 9**	***Survey 10***
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	***Year 10***
Assessment of RDT[4]	●	●	●	●	●	●	●	●	●	●
Study conclusion	●	●	●	●	●	●	●	●	●	●

Footnotes to Table 2:

● is used to indicate a study procedure that requires documentation in the individual eCRF

[1] ~~The n~~New sites will join the study after survey 3, depending on when they are included in EPI-MAL-002/-003. The total surveys at these new sites will therefore be less than ~~9~~***10***.

[2] RTS,S/AS01 and 3rd dose DTP/HepB/Hib and first dose measles EPI vaccinations, only

[3] Capillary blood sample for determination of parasite prevalence (blood slides and NAAT by filter paper)

[4] In the event of measured fever at time of visit (axillary temperature ≥37.5°C) or fever reported in last 24 hours or other symptoms/signs of clinical malaria, a rapid diagnostic test (RDT) will be conducted using capillary blood sample taken for blood slide preparation.

Table 3 List of study procedures to be conducted during survey

Epoch	**Epoch 001**	**Epoch 002**	**Epoch 003**	**Epoch 004**	**Epoch 005**	**Epoch 006**	**Epoch 007**	**Epoch 008**	**Epoch 009**	***Epoch 010***
Study Group	**Survey 1**	**Survey 2**	**Survey 3**	**Survey 4**	**Survey 5**	**Survey 6**	**Survey 7**	**Survey 8**	**Survey 9**	***Survey 10***
Timepoint	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	***Year 10***
Site specific information collection - Meteorological data [1]	●	●	●	●	●	●	●	●	●	●
Site specific information collection – Malaria Control Programme	●	●	●	●	●	●	●	●	●	●

● is used to indicate a study procedure that requires documentation in the individual eCRF.

[1] Availability of meteorological data will be based on the site having the weather station. All sites received weather stations either post Survey 1 or 2 or at study start for the new sites.

5.5.7. Collect data on malaria control interventions

Record the following malaria control measures in the subject's eCRF:

- ***Indoor*** ~~R~~***r***esidual spraying ***(IRS)***, mosquito net usage ***(including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN])***, seasonal malaria chemoprevention (SMC), intermittent preventive treatment in infants (IPTi), and all anti-malaria therapy received within the last 14 days.

5.5.10. Site specific information collection

…….

Centre specific information about interventions from the malaria control programme in the study area, such as bednet usage ***(including ITN and LLIN)***, IRS, ~~RDT,~~ ACT, SMC, IPTp and IPTi, will be collected by questionnaire and recorded in a separate database to that for subject-specific data.

7.1.1. Primary endpoints

- Occurrence of *P. falciparum* parasitaemia (using microscopy)

  Criteria/definitions: infection with *P. falciparum* determined using a blood smear slide and determined using microscopy.

- Occurrence of malaria control interventions

  Criteria/definitions: malaria control interventions are mosquito net usage ***(including insecticide-treated nets [ITN] and long lasting insecticidal nets [LLIN]), indoor*** residual spraying ***(IRS)***, seasonal malaria chemoprevention (SMC), intermittent preventative treatment in infants (IPTi), and ACT therapy received within the last 14 days.

7.2.1 Children aged 6 months to <5 years

…….

~~Up to seven centres will take part in this study.~~ ~~These~~ ~~centres~~ ***Study sites*** are in different endemicity classes, so a sample of 400 subjects in this age class will allow for suitable confidence limits around parasite prevalence estimates for sites within each of the endemicity classes.

7.7.1.2. By vaccine eligible/ineligible subgroup: Statistical analyses for vaccine association adjustments and association between vaccine and transmission

For the analysis of association between RTS,S/AS01$_E$ vaccine and transmission, two subgroups must be considered:

- Vaccine eligible subgroup (see glossary of terms for definition). ~~After introduction of the vaccine, there will be a catch-up for children less than 18 months old. Taking into account the 18 months of follow-up for the vaccine safety study, the oldest children vaccinated who could potentially be enrolled could be 3.5 years old.~~ This subgroup will be used to assess the association between vaccination and transmission intensity, assuming a constant MTI in unvaccinated subjects (which will be measured using the second subgroup)..

- Vaccine ineligible subgroup (see glossary of terms for definition). Due to the fact that the main reservoir of malaria is in the population between 5-20 years old (based on data collected in parallel [Epi-Mal-001 study] to the pivotal efficacy Phase III trial, not published) and MTI will be assessed for ~~up to 2~~ ***approximately 5*** years following the introduction of the vaccine, it will be assumed that there will be negligible herd immunity effect. Therefore, this subgroup will be used to detect any annual fluctuation (control group). If there is any herd effect, then the vaccine effect will be an underestimate.

~~Assuming a sample size of 600 subjects per centre, and per survey, and a similar sample size of children aged 0 to <5 years (JTEG-requested age and MTI reference for RTS,S/AS01 vaccine efficacy by research centre transmission intensity, pending Phase III data) and 2 to <10 years (WHO definition [WHO, 1963]); and trying to maximize the sample size of the 0-2 year subgroup, it is expected to have 240 subjects in the vaccine eligible subgroup and 360 subjects in the vaccine ineligible subgroup.~~

10. REFERENCES

International Society for Pharmacoepidemiology (ISPE). Guidelines for Good Pharmacoepidemiology Practices (GPP). Rev 3 (June 2015). http://www.pharmacoepi.org/resources/guidelines_08027.cfm; 2015. Accessed 14 June 2017.

Vaccines R & D

Protocol Administrative Change 1

eTrack study number and Abbreviated Title	116682 (EPI-MALARIA-005 BOD AME)
Administrative Change number:	Administrative Change 1 Final
Administrative Change date:	28 October 2021
Co-ordinating author:	PPD Freelance Science Writer for GSK Biologicals

Rationale/background for changes:

EPI-MALARIA-005 is referenced as a category 3 study in the Risk Management Plan (RMP) and was initially not classified as a PASS. In order to align with the GVP Module V Revision 2, where all category 3 studies assessing a risk are now classified as PASS, this study has been reclassified as a PASS.

This administrative change to the protocol has been put in place to add additional information to comply with the ENCePP Checklist for study protocols, comprising adding 'EU PAS Register No.', 'Product reference', 'Procedure number' and Joint PASS status on the title page, and dates for study milestones to 'Study Design'.

Additionally, according to EU regulation, the definition of end of study (EoS) must be included in the clinical protocol, and the study report submitted in a predefined timeframe based on the EoS milestone. Per GSK policies, a summary of the study results have to be publicly disclosed, the reference milestone for which is based on the primary completion date (PCD). The definitions for the EoS and PCD have been clarified.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Title page

Date of protocol amendment*/administrative change*	Amendment 1 Final: 21 September 2016 Amendment 2 Final: 14 September 2018 **Administrative Change 1 Final: 28 October 2021**
EU PAS Register No.	***EUPAS43920***
Product reference	***H0002300***
Procedure number	***Not allocated***

Joint PASS	***No***
Contributing authors	• PPD ***Study Delivery Lead***
	• PPD ***Senior Lead Epidemiologist-Malaria***

SYNOPSIS

Study design

- ***Primary completion date (PCD): the date of final collection of data for all primary outcomes/endpoints.***
- ***End of Study (EoS): the date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***

Milestones

Milestone	*Planned/Actual dates*
Start of data collection	*Q4 2014**
Last subject last visit (LSLV)	*Q3 2024*
End of data collection	*Q1 2025*
Interim report 1 (Surveys 1 & 2)	*Q3 2017**
Interim report 2 (Surveys 3, 4 & 5)	*Q1 2021**
Registration in the EU PAS register	*Q4 2021**
Final report of study results	*Q4 2025*

**Actual dates*

LIST OF APPENDICES

LIST OF ABBREVIATIONS

EoS	***End of Study***
LSLV	***Last Subject Last Visit***
PCD	***Primary Completion Date***

GLOSSARY OF TERMS

End of study (EoS):	***For studies with collection of Human Biologicals Samples, EoS is defined as:*** ***The date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***
Primary completion date (PCD):	***PCD is defined as the date of final collection of data for all primary outcomes/endpoints.***

3. STUDY DESIGN OVERVIEW

- ***Primary completion date (PCD): the date of final collection of data for all primary outcomes/endpoints.***
  Refer to GLOSSARY OF TERMS for the definition of primary completion date.
- ***End of Study (EoS): the date of the last testing/reading released of the Human Biological Samples related to primary and secondary endpoints. EoS must be achieved no later than 8 months after last subject last visit (LSLV).***
  Refer to GLOSSARY OF TERMS for the definition of EoS.
- ***Milestones***

Milestone	*Planned/Actual dates*
Start of data collection	*Q4 2014**
Last subject last visit (LSLV)	*Q3 2024*
End of data collection	*Q1 2025*
Interim report 1 (Surveys 1 & 2)	*Q3 2017**
Interim report 2 (Surveys 3, 4 & 5)	*Q1 2021**
Registration in the EU PAS register	*Q4 2021**
Final report of study results	*Q4 2025*

**Actual dates*

Annex 10 ENCePP Checklist for study protocols added

APPENDIX D ENCEPP CHECKLIST FOR STUDY PROTOCOLS

Section 1: Milestones	***Yes***	***No***	***N/A***	***Page Number(s)***
1.1 Does the protocol specify timelines for				
	☒	☐	☐	***14, 35***
1.1.1 Start of data collection	☒	☐	☐	***14, 35***
1.1.2 End of data collection	☐	☒	☐	
1.1.3 Study progress report(s)	☒	☐	☐	***14, 35***
1.1.4 Interim progress report(s)	☒	☐	☐	***14, 35***
1.1.5 Registration in the EU PAS register	☒	☐	☐	***14, 35***
1.1.6 Final report of study results.				

Section 2: Research question	***Yes***	***No***	***N/A***	***Page Number(s)***
2.1 Does the formulation of the research question and objectives clearly explain:				
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	***30-31***
2.1.2 The objective(s) of the study?	☒	☐	☐	***32***
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	***36-39*** ***58-60, 64***
2.1.4 Which formal hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no a priori hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	***Yes***	***No***	***N/A***	***Page Number(s)***
3.1 Is the study design described? (e.g. cohort, case-control, randomised controlled trial, new or alternative design)	☒	☐	☐	***33***
3.2 Does the protocol specify the primary and secondary (if applicable) endpoint(s) to be investigated?	☒	☐	☐	***52-54***
3.3 Does the protocol describe the measure(s) of effect? (e.g. relative risk, odds ratio, deaths per 1000 person-years, absolute risk, excess risk, incidence rate ratio, hazard ratio, number needed to harm (NNH) per year)	☒	☐	☐	***52-54***

Comments:

Section 4: Source and study populations	***Yes***	***No***	***N/A***	***Page Number(s)***
4.1 Is the source population described?	☒	☐	☐	***36-39***
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period?	☒	☐	☐	***34***
4.2.2 Age and gender?	☒	☐	☐	***39***
4.2.3 Country of origin?	☒	☐	☐	***37***
4.2.4 Disease/indication?	☒	☐	☐	***30, 31***
4.2.5 Co-morbidity?	☐	☐	☒	
4.2.6 Seasonality?	☒	☐	☐	***35***

4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	***39***

Comments:

Section 5: Exposure definition and measurement	***Yes***	***No***	***N/A***	***Page Number(s)***
5.1 Does the protocol describe how exposure is defined and measured? (e.g. operational details for defining and categorising exposure)	☐	☐	☒	
5.2 Does the protocol discuss the validity of exposure measurement? (e.g. precision, accuracy, prospective ascertainment, exposure information recorded before the outcome occurred, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure classified according to time windows? (e.g. current user, former user, non-use)	☐	☐	☒	
5.4 Is exposure classified based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.5 Does the protocol specify whether a dose-dependent or duration-dependent response is measured?	☐	☐	☒	

Comments:

Section 6: Endpoint definition and measurement	***Yes***	***No***	***N/A***	***Page Number(s)***
6.1 Does the protocol describe how the endpoints are defined and measured?	☒	☐	☐	***52-54***
6.2 Does the protocol discuss the validity of endpoint measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, prospective or retrospective ascertainment, use of validation sub-study)	☒	☐	☐	***54-56***

Comments:

Section 7: Confounders and effect modifiers	***Yes***	***No***	***N/A***	***Page Number(s)***
7.1 Does the protocol address known confounders? (e.g. collection of data on known confounders, methods of controlling for known confounders)	☒	☐	☐	***31, 42-44, 64***
7.2 Does the protocol address known effect modifiers? (e.g. collection of data on known effect modifiers, anticipated direction of effect)	☒	☐	☐	***31, 42-44, 64***

Comments:

Section 8: Data sources	***Yes***	***No***	***N/A***	***Page Number(s)***
8.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
8.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview, etc.)	☐	☐	☒	
8.1.2 Endpoints? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview	☒	☐	☐	***42-44***

including scales and questionnaires, vital statistics, etc.) ***8.1.3 Covariates?***	☐	☐	☒	
8.2 Does the protocol describe the information available from the data source(s) on: ***8.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)*** ***8.2.2 Endpoints? (e.g. date of occurrence, multiple event, severity measures related to event)*** ***8.2.3 Covariates? (e.g. age, gender, clinical and drug use history, co-morbidity, co-medications, life style, etc.)***	☐ ☒ ☐	☐ ☐ ☐	☒ ☐ ☒	***52-54***
8.3 Is a coding system described for: ***8.3.1 Diseases? (e.g. International Classification of Diseases (ICD)-10)*** ***8.3.2 Endpoints? (e.g. Medical Dictionary for Regulatory Activities (MedDRA) for adverse events)*** ***8.3.3 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC)Classification System)***	☐ ☐ ☐	☒ ☒ ☐	☐ ☐ ☒	
8.4 Is the linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☒	☐	

Comments:

Section 9: Study size and power	***Yes***	***No***	***N/A***	***Page Number(s)***
9.1 Is sample size and/or statistical power calculated?	☒	☐	☐	***54-56***

Comments:

Section 10: Analysis plan	***Yes***	***No***	***N/A***	***Page Number(s)***
10.1 Does the plan include measurement of excess risks?	☐	☒	☐	
10.2 Is the choice of statistical techniques described?	☒	☐	☐	***57-64***
10.3 Are descriptive analyses included?	☒	☐	☐	***57, 64***
10.4 Are stratified analyses included?	☐	☒	☐	
10.5 Does the plan describe methods for adjusting for confounding?	☒	☐	☐	***64***
10.6 Does the plan describe methods addressing effect modification?	☒	☐	☐	***64***

Comments:

Section 11: Data management and quality control	***Yes***	***No***	***N/A***	***Page Number(s)***
11.1 Is information provided on the management of missing data?	☐	☒	☐	
11.2 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	***66***
11.3 Are methods of quality assurance described?	☒	☐	☐	***66***
11.4 Does the protocol describe possible quality issues related to the data source(s)?	☒	☐	☐	***37, 38***
11.5 Is there a system in place for independent review of study results?	☐	☒	☐	

1 ***Comments:***

Section 12: Limitations	***Yes***	***No***	***N/A***	***Page Number(s)***
12.1 Does the protocol discuss:				
12.1.1 Selection biases?	☒	☐	☐	***37, 38***
12.1.2 Information biases?	☒	☐	☐	***37, 38***
(e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods)				
12.2 Does the protocol discuss study feasibility? (e.g. sample size, anticipated exposure, duration of follow-up in a cohort study, patient recruitment)	☒	☐	☐	***54-56***
12.3 Does the protocol address other limitations?	☐	☒	☐	

Comments:

Section 13: Ethical issues	***Yes***	***No***	***N/A***	***Page Number(s)***
13.1 Have requirements of Ethics Committee/Institutional Review Board approval been described?	☒	☐	☐	***39-40***
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☒	☐	
13.3 Have data protection requirements been described?	☒	☐	☐	***65-66***

Comments:

Section 14: Amendments and deviations	***Yes***	***No***	***N/A***	***Page Number(s)***
14.1 Does the protocol include a section to document future amendments and deviations?	☒	☐	☐	***74***

Comments:

Section 15: Plans for communication of study results	***Yes***	***No***	***N/A***	***Page Number(s)***
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	***67***
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	***67***

Comments:

1. ***Name of the main author of the protocol*: _____**

Date: / /

***Signature:* _____**

(Administrative Change 1, 28 October 2021)