NON-INTERVENTIONAL STUDY REPORT

TITLE PAGE

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Title:	A Phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
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STUDY INFORMATION

Title	A Phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
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Research question and objectives	Co-primary objectives: • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in participants aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$. • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in participants aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.
Countries of study	Ghana, Kenya
Author	Study Protocol Author: PPD Clinical and Epidemiology Research & Development Project Lead

MARKETING AUTHORISATION HOLDER

Marketing authorisation holder	GlaxoSmithKline Biologicals Rue de l'Institut 89, 1330 Rixensart, Belgium
MAH contact person	PPD PPD Global Regulatory Affairs GSK

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SPONSOR SIGNATORY

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Compound Number: SB257049

François Roman
Clinical and Epidemiology Research & Development
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Date (DD Month YYYY)

CO-SPONSOR SIGNATORY

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Compound Number: SB257049

Bronwyn MacInnis, Director Pathogen Genomic Surveillance
The Broad Institute

Date (DD Month YYYY)

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LIST OF ABBREVIATIONS

AE	Adverse Event
AMC	Academic Medical Center
$AS01_E$	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome
BI	Broad Institute
CI	Confidence Interval
CIGAR	Compact Idiosyncratic Gapped Alignment Report
CS	Circumsporozoite
CSP	Circumsporozoite Protein
CQA	Clinical development quality assurance
GH	Ghana
GQC	Global quality compliance
GSK	GlaxoSmithKline Biologicals
HBsAg	Hepatitis B surface antigen
HSPH	Harvard T. H. Chan School of Public Health
IC	Informed Consent
ICF	Informed consent form
IR	Interim Report
KE	Kenya
LAR	Legally Authorized Representative
MVIP	Malaria Vaccine Implementation Program
N	Total number of eligible participants
n	Number of participants in a given category
NAAT	Nucleic Acid Amplification Test

OR	Odds Ratio
PCD	Primary Completion Date
P. falciparum	*Plasmodium falciparum*
R&D	Research & Development
RTS	Hybrid protein comprising HBs (hepatitis B surface antibody) and CS protein portions
RTS,S	Particulate antigen, containing both RTS and HBs antigen (S) proteins
SAP	Statistical Analysis Plan
SD	Standard Deviation
SERA2	Serine Repeat Antigen 2
SSA	Sub-Saharan Africa
TMF	Trial Master File
WHO	World Health Organisation

TRADEMARK INFORMATION

Trademarks of the GSK group of companies	Trademarks not owned by the GSK group of companies
NA	NA

1. RESPONSIBLE PARTIES

GSK has the overall responsibility for the conduct of the study.

PPD (Epidemiology Lead – Malaria) and PPD Epidemiology) are the GSK-designated contact persons for this study.

1.1. Study Advisory Committee

No advisory committee was used in this study.

2. SYNOPSIS

Title

A Phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.

Keywords

RTS,S/AS01$_E$, malaria, Sub-Saharan Africa, genetic diversity, *Plasmodium falciparum*

Rationale and background

GlaxoSmithKline Biologicals (GSK) has developed a pre-erythrocytic *Plasmodium (P.) falciparum* malaria vaccine, RTS,S/AS01$_E$, for routine immunization of infants and children living in malaria-endemic countries of Sub-Saharan Africa (SSA). The vaccine antigen, RTS,S, is comprised of RTS (fusion protein containing the repeat portion and the C-terminus of the circumsporozoite [CS] protein of the clone 3D7, derived from the *P. falciparum* NF54 strain, and the amino terminal end of the hepatitis B surface antigen [HBsAg]) and of HBsAg co-expressed in *Saccharomyces cerevisiae* yeast expression system.

The pre-authorization clinical development has been conducted mainly in SSA countries. The main clinical study supporting the efficacy and safety is the large Phase 3 study, MALARIA-055 [110021]. Following this study, 2 consecutive studies (EPI-MAL-002 is completed and EPI-MAL-003 is ongoing) are being conducted to monitor incidence rates of meningitis, protocol-defined adverse events of special interest and other adverse events (AEs) leading to hospitalizations and death.

In parallel with both the EPI-MAL-002 and EPI-MAL-003 studies, EPI-MAL-005 was conducted to measure malaria transmission intensity and the use of other malaria control interventions such as residual spraying and bed nets on a yearly basis during the rainy season.

This ancillary study to EPI-MAL-005, referred to as the EPI-MAL-010 study, monitored the genetic diversity in CS sequences in the *P. falciparum* parasite population before and after vaccine implementation in children aged 6 months to <5 years.

Research questions and objectives

Co-primary objectives:

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in participants 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in participants aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.

These co-primary objectives involved pharmacogenomics testing.

Secondary objectives:

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in participants aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in participants aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To estimate trends in longitudinal prevalence of *P. falciparum* in participants aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To estimate trends in longitudinal frequency of *P. falciparum* in participants aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.

These secondary objectives involved pharmacogenomics testing.

Study design

- Type of design: Longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study. In order to characterize *P. falciparum* haplotypes, genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, 1 site in Eastern Africa and 1 site in Western Africa. Those sites were the same during the entire duration of the study.
- Study population: Participants aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at the 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, were included in the EPI-MAL-010 study.
- Biological samples: In the EPI-MAL-005 study, a blood sample was obtained for malaria blood slide reading conducted locally, and 2 to 3 drops of blood were spotted onto filter paper for the Nucleic Acid Amplification Test (NAAT) (conducted at Academic Medical Centre [AMC], Amsterdam, The Netherlands). Both blood slide reading (by microscopy) and NAAT (from genomic deoxyribonucleic acid [DNA]) were used to evaluate the level of asexual *P. falciparum* parasitemia. The

EPI-MAL-010 study re-used DNA samples collected and processed during the EPI-MAL-005 study. For samples that were identified as positive for *P. falciparum* by malaria blood slide reading and/or by NAAT, a minimum of 15 microliters of DNA extracted at AMC were sent to Harvard T. H. Chan School of Public Health (HSPH)/ Broad Institute (BI) for amplicon sequencing.

- Sampling schedule: The EPI-MAL-010 study re-used samples from participants enrolled in 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study. Selection of surveys included in EPI-MAL-010 was contingent upon the start date of the Malaria Vaccine Implementation Programme (MVIP) and required the first surveys to occur prior to MVIP start.
- Primary completion date: PCD was defined as the date of final collection of data for all primary outcomes.
- End of study: Last testing results released of samples re-used from EPI-MAL-005. End of study must be achieved no later than 8 months after the selection of the samples for testing of the last survey.
- Duration of the study: This design allowed monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after the start of RTS,S/AS01$_E$ vaccination, across 7 consecutive annual surveys as described below:
  - 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

  The numbering of surveys starts from Survey 1 to 7 in the protocol. However, this was updated as Survey 3 to Survey 9 to be aligned with the numbering of the EPI-MAL-005 study, to facilitate cross-referencing between both studies.

Setting

DNA samples collected and processed during the EPI-MAL-005 study were reused for the EPI-MAL-010 study. Only positive samples for *P. falciparum* infection were sent to HSPH/BI for sequencing.

In the framework of the MVIP, the RTS,S/AS01$_E$ vaccine was planned to be administered by the Ministry of Health through the Expanded Programme on Immunization as a three-dose primary series, followed by a fourth booster dose. Based on this information and on the current assumption that the first three doses of RTS,S/AS01$_E$ would be administered to all participants within a period of 18 months, conducting the EPI-MAL-010 study during 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study, pre- and post-RTS,S/AS01$_E$ MVIP start, allowed monitoring

haplotype diversity in a representative proportion of participants having received the vaccine.

Participants and study size

Participants aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, were included in the EPI-MAL-010 study.

In the MVIP, 3 countries (Ghana, Kenya, and Malawi) were selected for the vaccine implementation in EPI-MAL-003 study, and 2 of these countries (Ghana and Kenya) were selected for participation in the EPI-MAL-010 study. The study sites were selected from these 2 countries where the RTS,S/AS01$_E$ vaccine was to be implemented: Ghana and Kenya. Sites from Ghana and Kenya were moderate-to-high transmission areas. Based on the results from MALARIA- 066, selecting 1 site situated in Western Africa and the other in Eastern Africa allows further exploration of the previously observed *P. falciparum* strain diversity differences between Western and Eastern Africa, and increases the continental diversity of study participants in EPI-MAL-010.

Each EPI-MAL-005 site aimed to enroll about 600 children aged 6 months to 10 years per annual cross-sectional survey, of which about 400 children were aged 6 months to <5 years according to stratification by age group as follows (all participant numbers were 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

Therefore, the total expected sample size per survey and per site was a maximum of 400 participants aged 6 months to <5 years.

Variables and data sources

Primary endpoint

- Occurrence of specific *P. falciparum* haplotype infection.
  - Criteria/definitions: infection with (a) particular *P. falciparum* haplotype(s) confirmed for the CS C-terminus and/or Serine Repeat Antigen 2 (SERA2) loci using sequencing techniques. Based on recommendation by the testing laboratory, infection was confirmed using CS C-terminus for sequencing.

Secondary endpoints

Secondary endpoints were identical to the primary endpoint. For trends analyses, only the 3D7 haplotype and the haplotypes with more than 5% of frequency were considered.

Data sources

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study.

In order to determine *P. falciparum* haplotypes, genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, 1 site in Eastern Africa and 1 site in Western Africa.

Moreover, information regarding demographic data, vaccinations status, blood sampling, etc., was obtained using an extraction from the EPI-MAL-005 database for all the participants enrolled in EPI-MAL-010.

Results

Disposition of participants:

Participants in the Analysis set were distributed over 2 centers (Kintampo and Kombewa) in 2 countries (Ghana [GH], Kenya [KE]) respectively.

The numbering of surveys starts from Survey 1 to 7 in the protocol. However, this was updated as Survey 3 to Survey 9 to be aligned with the numbering of the EPI-MAL-005 study, to facilitate cross-referencing between both studies.

The Analysis set (inclusive of both sites) for each survey included 801 participants (Survey 3), 796 participants (Survey 4), 801 participants (Survey 5), 801 participants (Survey 6), 799 participants (Survey 7), 800 participants (Survey 8), and 801 participants (Survey 9). Out of the total number of enrolled participants at both sites, and those who were eligible for sample analysis (positive for *P. falciparum* by malaria blood slide reading and/or by NAAT), the proportion of participants with valid samples analyzed and total number of detected haplotypes with cut-off of 15 reads were as follows:

- Survey 3: out of the 339 eligible samples, 270 samples were successfully analyzed across both sites (79.1% in Kintampo and 80.1% in Kombewa). The overall number of detected haplotypes were 340 and 361, respectively.
- Survey 4: out of the 326 eligible samples, 262 samples were successfully analyzed across both sites (80.3% in Kintampo and 80.5% in Kombewa). The overall number of detected haplotypes were 294 and 364, respectively.
- Survey 5: out of the 278 eligible samples, 195 samples were successfully analyzed across both sites (64.2% in Kintampo and 73.8% in Kombewa). The overall number of detected haplotypes were 121 and 297, respectively.

- Survey 6: out of the 254 eligible samples, 209 samples were successfully analyzed across both sites (88.0% in Kintampo and 79.0% in Kombewa). The overall number of detected haplotypes were 219 and 358, respectively.
- Survey 7: out of the 203 eligible samples, 157 samples were successfully analyzed across both sites (74.3% in Kintampo and 79.1% in Kombewa). The overall number of detected haplotypes were 85 and 245, respectively.
- Survey 8: out of the 170 eligible samples, 120 samples were successfully analyzed across both sites (63.6% in Kintampo and 73.0% in Kombewa). The overall number of detected haplotypes were 64 and 206, respectively.
- Survey 9: out of the 167 eligible samples, 109 samples were successfully analyzed across both sites (63.3% in Kintampo and 66.1% in Kombewa). The overall number of detected haplotypes were 71 and 168, respectively.

Among participants who tested positive for *P. falciparum*, the total number of circumsporozoite protein (CSP) haplotypes detected for a particular survey at each site showed a decrease after implementation of the vaccination program at both sites. In parallel, the proportion of samples that failed to be sequenced fluctuated across surveys and were high in Surveys 8 and 9 for both sites, which could have contributed to observed reduction in the total number of detected haplotypes in more these surveys.

Demographic characteristics:

GH Kintampo: The mean (standard deviation [SD]) age at informed consent (IC) for the different surveys were 2.32 (1.192) years (Survey 3), 2.33 (1.165) years (Survey 4), 2.39 (1.130) years (Survey 5), 2.29 (1.164) years (Survey 6), 2.31 (1.156) years (Survey 7), 2.28 (1.187) years (Survey 8), and 2.31 (1.175) years (Survey 9). Overall, there were more male participants than female in all the surveys except for Surveys 8 and 9.

KE Kombewa: The mean (SD) age at IC for the different surveys were 2.36 (1.184) years (Survey 3), 2.34 (1.188) years (Survey 4), 2.33 (1.221) years (Survey 5), 2.33 (1.183) years (Survey 6), 2.40 (1.191) years (Survey 7), 2.30 (1.193) years (Survey 8), and 2.38 (1.192) years (Survey 9). Overall, there were more female participants than male in all surveys except for Surveys 5 and 9.

Vaccination history of RTS,S/AS01$_E$:

Details regarding Survey 6 and Survey 7 are presented in the Interim Report (IR), dated 26 November 2024. Surveys 3, 4, and 5 were conducted before the start of RTS,S/AS01$_E$ vaccination and Surveys 6, 7, 8, and 9 were conducted after the start of RTS,S/AS01$_E$ vaccination.

GH Kintampo:

- Out of the 398 participants from GH Kintampo during Survey 8, overall 64.3% participants were vaccinated (with at least one dose of RTS,S/AS01$_E$). The majority of participants in the 0.5 to 1 year age group had received 3 doses (64.0%), while the majority in the 2 to 4 years age group had not been vaccinated (54.1%).

- Out of the 400 participants from GH Kintampo during Survey 9, overall 75.0% participants were vaccinated (with at least one dose of RTS,S). The majority of participants in the 0.5 to 1 year age group had received 3 doses (57.4%). While the majority in the 2 to 4 years age group had received 4 doses (36.2%), a similar proportion (32.6%) had not been vaccinated.

KE Kombewa:

- Out of the 402 participants from KE Kombewa during Survey 8, overall 59.5% participants were vaccinated (with at least one dose of RTS,S/AS01$_E$). The majority of participants in the 0.5 to 1 year age group had received 3 doses (57.9%), while the majority in the 2 to 4 years age group had not been vaccinated (51.6%) and a lower proportion (28.3%) in the 2 to 4 years age group had received 4 doses.
- Out of the 401 participants from KE Kombewa during Survey 9, overall 76.1% participants were vaccinated (with at least one dose of RTS,S/AS01$_E$). The majority of participants in the 0.5 to 1 year age group had received 3 doses (74.6%), while the majority in the 2 to 4 years age group had not been vaccinated (36.4%) and a similar proportion (31.8%) in the 2 to 4 years age group had received 4 doses.

Co-Primary and Secondary objective results:

All primary and secondary analyses on prevalence and frequency were reported by site and per survey. For these analyses, a threshold of 15 reads per haplotype within a sample was used. This threshold originated from MALARIA-066 study where positive samples were rich with parasite genetic materials. Based on MALARIA-095 study where more updated sequencing protocol was followed compared to MALARIA-066 study, the threshold point of 50 reads was considered more accurate. Therefore, a sensitivity analysis on a threshold of 50 reads was provided. The analyses were performed on the 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, the 10 most frequently detected haplotypes were presented.

Longitudinal Prevalence:

- 3D7 haplotype prevalence at GH Kintampo overall, was highest during Survey 3 (6.23%; Confidence Interval [CI]: 4.07 – 9.07) and showed a gradual decline to Survey 9 (0.25%; CI: 0.01 – 1.38) and was not detected during Survey 8. Among female participants, 3D7 haplotype prevalence was highest during Survey 3 (6.95%; CI: 3.75 – 11.59), while among male participants, it was highest in Survey 4 (6.45%; CI: 3.57 – 10.59) and was not detected during Survey 8 and 9. Among 0.5 to 1 year old participants, 3D7 haplotype prevalence was highest during Surveys 3 and 4 (3.85%; CI: 1.56 - 7.76, each) and not detected during Surveys 7, 8, and 9, whereas among 2 to 4 years old participants, it was highest during Survey 3 (8.22%; CI: 4.94 - 12.68) and not detected during Survey 8. Among unvaccinated participants, 3D7 haplotype prevalence was highest during Survey 3 (6.23%; CI: 4.07 - 9.07) and not detected during Survey 8, while 3D7 haplotype was not detected among vaccinated participants during Surveys 6, 7, 8, and 9.
- 3D7 haplotype prevalence in KE Kombewa fluctuated across surveys overall, where the highest prevalence was observed during Survey 9 (1.75%; CI: 0.7 – 3.56) and was not detected during Survey 7 (0%; CI: 0 – 0.92). Among female participants in

KE Kombewa, 3D7 haplotype prevalence was highest during Survey 3 (1.88%; CI: 0.51 - 4.74) and not detected during Survey 7, while among male participants, it was highest in Survey 9 (1.98%; CI: 0.54 - 4.99) and not detected during Surveys 3, 4, and 7. Among 0.5 to 1 year old participants, 3D7 haplotype prevalence was highest in Survey 9 (2.76%; CI: 0.9 - 6.33) and was not detected in Surveys 6 and 7, while among 2 to 4 years old participants it was highest in Surveys 3 and 5 (1.37%; CI: 0.28 - 3.95, each) and not detected in Surveys 4 and 7. Among unvaccinated participants, 3D7 haplotype prevalence was highest during Survey 8 (1.84%; CI: 0.38 - 5.28) and not detected during Survey 7, while among vaccinated participants it was 1.97% (CI: 0.73 - 4.23) during Survey 9, but was not detected during Surveys 6, 7, and 8.

- Haplotype CIG32 showed the highest prevalence at GH Kintampo overall, during Survey 4 (8.29% [CI: 5.78 - 11.45]) and most prevalent across Survey 5 (2.76%; CI: 1.38 - 4.88), Survey 6 (5.72%; CI: 3.66 - 8.46), Survey 7 (2.26%; CI: 1.04 - 4.24), and Survey 8 (3.02% CI: 1.57 - 5.21). CIG32 was the most prevalent haplotype across most surveys in female participants (all except Survey 3) as well as among male participants (all except Surveys 3, 5, and 9). Among 0.5 to 1 year old participants, CIG32 was the most prevalent haplotype across most surveys (Surveys 4, 6, 7, 8, and 9), while CIG39 was the most prevalent haplotype across Surveys 3, 5, and 9. Among 2 to 4 years old participants, CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8. CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8 among unvaccinated participants, whereas among the participants who received RTS,S/AS01$_E$ vaccination, CIG32 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG38 was most prevalent across Surveys 7 and 9.
- Haplotype CIG32 was the most prevalent haplotype overall, across Survey 3 (9.25%; CI: 6.6 - 12.52), Survey 4 (11.31%; CI: 8.37 - 14.84), Survey 6 (10.78%; CI: 7.91 - 14.24), and Survey 9 (3.74%; CI:2.11 - 6.09) at KE Kombewa. Additionally, haplotype CIG33 showed >5% prevalence in all surveys except Surveys 8 and 9 at KE Kombewa (with highest prevalence in Survey 5 [8.46%; CI: 5.93 -11.62], Survey 7 [6.00%; CI: 3.88 - 8.8], and Survey 9 [3.74%; CI: 2.11 - 6.09]). Among female participants in Kombewa (Kenya), CIG32 was the most prevalent haplotype during Surveys 3, 4, 6, and 7, while CIG33 was the most prevalent during Surveys 5, 7, and 9. Among male participants in Kombewa (Kenya), CIG32 was the most prevalent haplotype across Surveys 3, 4, 5, and 9, while CIG33 was the most prevalent haplotype across Surveys 6 and 7. Among 0.5 to 1 year old participants, CIG32 was the most prevalent during Surveys 3, 4, 5, 7, and 9, whereas among 2 to 4 years old participants, CIG32 was the most prevalent haplotype across Surveys 3, 4, and 6, while CIG33 was the most prevalent haplotype across Surveys 5, 7, and 9. Among unvaccinated participants, CIG32 was the most prevalent haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most prevalent during Surveys 5, 7, and 9, whereas among vaccinated participants, CIG33 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG32 was most prevalent during Surveys 7 and 9.

Sensitivity analysis for prevalence:

- 3D7 haplotype prevalence under worst-case scenario, was highest during Survey 3 (14.46%; CI: 11.17 - 18.29) and lowest during Survey 8 (4.02%; CI: 2.32 - 6.45) at

GH Kintampo, while 3D7 haplotype prevalence was highest in Survey 5 (12.19%; CI: 9.16 - 15.79) and lowest in Survey 7 (6.75%; CI: 4.5 - 9.67) at KE Kombewa.

- Under the "worst case scenario" assumption for sensitivity analysis for prevalence at each time point, CIG32 was the most prevalent haplotype in Survey 4 (15.83%; CI: 12.38 - 19.79), Survey 5 (12.28%; CI: 9.23 - 15.91), Survey 6 (8.46%; CI: 5.93 - 11.62), Survey 7 (7.02%; CI: 4.71 - 9.98), and Survey 8 (7.04%; CI: 4.73 - 10.01) in GH Kintampo, while in KE Kombewa, the most prevalent haplotypes were CIG32 (Survey 3 [18.25%; CI: 14.59 - 22.39], Survey 4 [19.85%; CI: 16.04 - 24.11], Survey 6 [19.30%; CI: 15.54 - 23.52], and Survey 9 [13.72%; CI: 10.5 - 17.48]) and CIG33 (Survey 5 [19.65%; CI: 15.88 - 23.88], Survey 7 [12.75%; CI: 9.64 - 16.42], and Survey 9 [13.72%; CI: 10.5 - 17.48]).
- 3D7 haplotype prevalence, using a threshold of 50 reads was highest during Survey 3 (5.99%; CI: 3.87 - 8.77) and was not detected during Survey 8 at GH Kintampo; while 3D7 haplotype prevalence was highest in Survey 9 (1.50%; CI: 0.55 - 3.23) and was not detected during Survey 7 at KE Kombewa.
- Using a threshold of 50 reads, CIG32 haplotype showed highest prevalence across Survey 4 (7.79%; CI: 5.35 - 10.87), Survey 5 (2.76%; CI: 1.38 - 4.88), Survey 6 (5.47%; CI: 3.46 - 8.17), Survey 7 (2.26%; CI: 1.04 - 4.24), and Survey 8 (3.02%; CI: 1.57 - 5.21) in GH Kintampo as well as Survey 3 (8.75%; CI: 6.17 - 11.96), 4 (9.80%; CI: 7.06 - 13.15), Survey 6 (10.53%; CI: 7.69 - 13.96), and Survey 9 (3.49%; CI: 1.92 - 5.79) in KE Kombewa.

Longitudinal frequency:

- Haplotype 3D7 showed highest frequency overall, during Survey 7 (9.41%; CI: 4.15 - 7.71) and was not detected during Survey 8 at GH Kintampo. Among female participants, 3D7 haplotype frequency was highest in Survey 3 (8.67%; CI: 4.7 - 14.36) and was not detected during Survey 8, whereas among male participants, it was highest in Survey 7 (10.64%; CI: 3.55 - 23.1) and not detected during Surveys 8 and 9. Among 0.5 to 1 year old participants, 3D7 haplotype frequency was highest during Survey 3 (7.95%; CI: 3.26 - 15.7) and not detected during Surveys 7, 8, and 9, while among 2 to 4 years old participants, it was highest during Survey 7 (11.94%; CI: 5.3 - 22.18) and not detected during Survey 8. Among unvaccinated participants, 3D7 haplotype frequency was highest during Survey 7 (10.67%; CI: 4.72 - 19.94) and not detected during Survey 8, whereas it was not detected among vaccinated participants (Surveys 6, 7, 8, and 9).
- Overall, 3D7 haplotype showed highest frequency during Survey 9 (4.17%; CI: 1.69 - 8.4) and was not detected during Survey 7 at KE Kombewa. Among female participants, 3D7 haplotype frequency was highest in Survey 9 (3.75%; CI: 0.78 – 10.57) and not detected during Survey 7, whereas among male participants it was highest in Survey 9 (4.55%; CI: 1.25 – 11.23) and not detected during Surveys 3, 4, and 7. Among 0.5 to 1 year old participants, the 3D7 haplotype frequency was highest in Survey 9 (7.58%; CI: 2.51 - 16.8) and not detected in Surveys 6 and 7, while among 2 to 4 years old participants, it was highest in Survey 9 (1.96%; CI: 0.24 – 6.9) and not detected in Surveys 4 and 7. Among unvaccinated participants, 3D7 haplotype frequency was highest during Survey 8 (3.16%; CI: 0.66 – 8.95) and not detected during Survey 7, while among

vaccinated participants it was 6.19% (CI: 2.3 – 12.98) during Survey 9 and not detected during Surveys 6, 7, and 8.

- Haplotype CIG32 was the most frequently detected haplotype in GH Kintampo across most surveys, overall (Survey 4 [11.22%; CI: 7.85 - 15.4], Survey 5 [9.09%; CI: 4.63 - 15.68], Survey 6 [10.50%; CI: 6.77 - 15.34], Survey 7 [10.59%; CI: 4.96 - 19.15], and Survey 8 [18.75%; CI: 10.08 – 30.46]). CIG32 was the most frequently detected haplotype across most surveys among female participants (Surveys 4, 5, 6, 7, 8, and 9) as well as among male participants (Surveys 4, 6, 7, and 8). CIG32 was also the most frequently detected haplotype among 0.5 to 1 year old participants (all surveys except for Surveys 3 and 5) as well as among 2 to 4 years old participants (Surveys 4, 5, 6, and 8). Considering vaccination status, CIG32 was the most frequently detected haplotype across most surveys among unvaccinated participants (Surveys 4, 5, 6, and 8), whereas among vaccinated participants, CIG32 was most frequently detected across Surveys 7 and 8, while CIG38 was most frequently detected across Surveys 7 and 9.
- Haplotype CIG32 was the most frequently detected haplotype across most surveys overall, in KE Kombewa (Survey 3 [10.25%; CI: 7.32 - 13.85], Survey 4 [12.36%; CI: 9.16 - 16.19], Survey 6 [12.01%; CI: 8.83 - 15.84], and Survey 9 [8.93%; CI: 5.08 - 14.3]). CIG32 was the most frequently detected haplotype across most surveys among female participants (Surveys 3, 4, 6, and 7) as well as male participants (Surveys 3, 4, 5, and 9). Among 0.5 to 1 year old participants, CIG32 was the most prevalent during most surveys (Surveys 3, 4, 5, 7, and 9). However, among 2 to 4 years old participants, CIG32 was the most prevalent haplotype across Surveys 3, 4, and 6, while CIG33 was the most prevalent haplotype across Surveys 5, 7, 8, and 9. Among unvaccinated participants, CIG32 was the most frequently detected haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most frequently detected during Surveys 5, 7, and 9. Among vaccinated participants, CIG33 was the most frequently detected haplotype across Surveys 6, 7, and 8.

Sensitivity analysis for frequency:

- 3D7 haplotype frequency using a threshold of 50 reads was highest during Survey 7 (8.64%) and was not detected during Survey 8 at GH Kintampo; while 3D7 haplotype frequency was highest in Survey 9 (3.77%) and was not detected during Survey 7 at KE Kombewa.
- Using a threshold of 50 reads, CIG32 haplotype had highest frequency across Survey 4 (11.40%; CI: 7.88 - 15.79), Survey 5 (10.68%; CI: 5.45 - 18.31), Survey 6 (10.09%; CI: 6.43 - 14.88), Survey 7 (11.11%; CI: 5.21 - 20.05), and Survey 8 (19.05%; CI: 10.25 - 30.91) at GH Kintampo as well as Survey 3 (10.39%; CI: 7.34 - 14.15), Survey 4 (11.27%; CI: 8.14 - 15.09), Survey 6 (12.14%; CI: 8.89 - 16.05), and Survey 9 (8.81%; CI: 4.9 - 14.33) at KE Kombewa.

Longitudinal prevalence trends for selected P. falciparum haplotypes:

Trends analysis was performed for 3D7 haplotype and haplotypes with ≥5% frequency across all 7 surveys, by site and according to RTS,S/AS01$_E$ vaccination status.

- In the overall population in GH Kintampo, significant ORs of <1.0 for *P. falciparum* 3D7 haplotype were estimated on Surveys 5 to 9 (OR = 0.387 at Survey 5,

OR = 0.384 at Survey 6, OR = 0.308 at Survey 7, and OR = 0.038 at Survey 9) compared to Survey 3, at statistical significance level of 0.05. A decrease in prevalence was observed from Survey 3 until Survey 9 with no case detected at Survey 8. No significant OR was estimated on surveys in the overall population in KE Kombewa.

- A significant OR of <1.0 for *P. falciparum* CIG32 haplotype prevalence was observed for the overall population during Surveys 7 and 9 (OR = 0.398 and OR = 0.130, respectively) compared to Survey 3, at statistical significance level of 0.05 in GH Kintampo. Significant ORs of *P. falciparum* CIG32 haplotype prevalence were detected at Surveys 7, 8 and 9 (OR = 0.571, OR = 0.250, OR = 0.381 respectively) compared to Survey 3 in the overall population in KE Kombewa.
- A significant OR of <1.0 for *P. falciparum* CIG33 haplotype prevalence was detected at Survey 5 through 9 (OR = 0.276, OR = 0.462, OR = 0.370, OR = 0.184, and OR = 0.183, respectively) compared to Survey 3, at statistical significance level of 0.05 in the overall population in GH Kintampo. A significant OR for *P. falciparum* CIG33 haplotype prevalence was noted during Surveys 8 and 9 (OR = 0.380 and OR = 0.479, respectively) compared to Survey 3 in the overall population in KE Kombewa.
- CIG35 haplotype was not detected with ≥5% prevalence across all surveys at GH Kintampo, while no significant OR was estimated on surveys in the overall population, in KE Kombewa.
- A significant OR of <1.0 for P. *falciparum* CIG38 haplotype prevalence was observed for Surveys 5 through 9 (OR = 0.347, OR = 0.463, OR = 0.191, OR = 0.038, and OR = 0.190, respectively) compared to Survey 3, at statistical significance level of 0.05 for the overall population in GH Kintampo. A significant OR of *P. falciparum* CIG38 haplotype prevalence was observed at Survey 9 (OR = 0.368) compared to Survey 3 in the overall population in KE Kombewa.
- A significant OR of <1.0 for P. *falciparum* CIG39 haplotype prevalence was observed for Surveys 5 through 9 (OR = 0.296, OR = 0.260, OR = 0.196, OR = 0.032, and OR = 0.097, respectively) compared to Survey 3, at statistical significance level of 0.05 for the overall population and the unvaccinated population in GH Kintampo, but no significant OR was estimated on surveys in KE Kombewa.
- The analyses on pre-breakpoint group of surveys and on post-breakpoint group of surveys showed a linear decrease for 3D7 haplotype at GH Kintampo (OR on before and after breakpoint groups), observed in the unadjusted model. However, when the OR was adjusted for variables: gender, age group, RTS,S/AS01$_E$ vaccination status and multiple infection, the results were not significant. This could indicate that the observed decrease in ORs is potentially a correlation between variables and not necessarily due to a decrease over time. For KE Kombewa, we observed that the post-breakpoint group was at Survey 7, which could be influenced by the higher prevalence of 3D7 haplotype in Survey 9 compared to prevalence of 3D7 haplotype in other surveys. However, the limitation here is that the model was forced to use this breakpoint as this was the survey when vaccination started. When there were several breakpoints, the first one given by the model would be the one around Survey 7.

- Multivariable piecewise logistic regressions (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) revealed for all the most frequent haplotypes that a multiple infection could be considered as a risk factor of infection for the most frequent haplotypes at KE Kombewa and KH Kintampo.

Discussion

EPI-MAL-010 was a longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study, where genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination.

3D7 haplotype was not detected among vaccinated cohort in both KE Kombewa (except in Survey 9) and GH Kintampo. However, it was observed even before vaccination started in S6 that prevalence of 3D7 was in decline in GH Kintampo. The trend analysis did not show significantly increased risk of infection with non-3D7 haplotypes. Therefore, the common concern about potential escape of the parasite to evade the immune response by a mismatching haplotype to 3D7 haplotype, that could compromise vaccine efficacy, was not supported.

Conclusions

This study monitored the genetic diversity of CS sequences in the *P. falciparum* parasite population (measured through both haplotype frequency and prevalence) before and after vaccine implementation in children aged 6 months to <5 years in both GH Kintampo and KE Kombewa. The number of detected haplotypes varied between the sites. Across the surveys, it was observed that number of observed haplotypes was declining, however this observation is confounded by the high sequencing failure rate in more recent surveys (up to 30%). The observed prevalence of detected *P. falciparum* haplotypes varied by site over the surveys. The observed frequency of detected *P. falciparum* haplotypes also varied by site over the surveys. There were other haplotypes, like CIG32, CIG33, and CIG38, whose prevalence (and frequency) fluctuated across surveys, however no clear trend could be observed. In general, no haplotype showed clear dominance over time, rather varying prevalence over time and no clear pattern observed in unvaccinated versus vaccinated children. Overall, the prevalence and frequency of detected 3D7 haplotype was higher in GH Kintampo across surveys compared to KE Kombewa. In particular, 3D7 prevalence varied from around 6% in Survey 3 among unvaccinated in Ghana down to 0.25% in Survey 9, and upward from 0% in Survey 7 to 1.8% in Survey 8 in the unvaccinated in Kenya. Given the study limitations that include the lack of adjustment to transmission period related factors including the length of rain season and other anti-malaria control interventions, caution is advised when generalizing the findings to other settings or populations.

Marketing authorization holder

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Names and affiliations of principal investigators

Not applicable.

3. AMENDMENTS AND UPDATES

None.

4. MILESTONES

Milestone	Planned date	Actual Date	Comments
Start of sample selection	Q3 2021	8 October 2021	-
End of data collection	Q3 2024	31 October 2024	End of data collection meaning when all results/data were received from Harvard/BI
Registration in the EU PAS register	Q3 2021	6 October 2021	-
Interim report	Q1 2023	26 November 2024	-
Final report of study results	Q2 2025	23 July 2025	-

5. RATIONALE AND BACKGROUND

5.1. Background

GSK has developed a pre-erythrocytic *P. falciparum* malaria vaccine, RTS,S/AS01$_E$, for routine immunization of infants and children living in malaria-endemic countries of SSA. RTS,S/AS01$_E$ is the first vaccine to be implemented for the prevention of malaria and the first AS01-adjuvanted vaccine to be implemented in the pediatric population.

The vaccine antigen, RTS,S, is comprised of RTS (fusion protein containing the repeat portion and the C-terminus of the CS protein of the clone 3D7, derived from the *P. falciparum* NF54 strain, and the amino terminal end of the HBsAg) and of HBsAg co-expressed in *Saccharomyces cerevisiae* yeast expression system.

The pre-authorization clinical development has been conducted mainly in SSA countries. The main clinical study supporting efficacy and safety is the large Phase 3 study, MALARIA-055 [110021], conducted at 11 study sites in 7 countries across SSA, which enrolled 15 459 children. In summary, a moderate protective efficacy against clinical disease and severe malaria that wanes over time was shown in the large Phase 3 study [Early, 2018; Neafsey, 2015].

5.2. Rationale

The safety profile of RTS,S/AS01$_E$ has been evaluated in clinical trials conducted mainly in SSA and was further monitored and evaluated during the MVIP. GSK has developed a Post Approval Plan comprised of a set of complementary prospective studies (EPI-MAL-005 study, EPI-MAL-002 study and EPI-MAL-003 study) to be conducted in similar if not identical settings in order to expand the data on vaccine safety, effectiveness and impact of the RTS,S/AS01$_E$ vaccine.

Following the pivotal Phase 3 study of the candidate malaria vaccine RTS,S/AS01$_E$ (MALARIA-055), 2 consecutive studies (EPI-MAL-002 and EPI-MAL-003) were conducted to monitor incidence rates of meningitis, protocol-defined adverse events of special interest and other AEs leading to hospitalizations and death. The first study, EPI-MAL-002, which started in Q4 2015, is a surveillance study before RTS,S/AS01$_E$ vaccination; the second study, EPI-MAL-003, which started in Q1 2019, monitors RTS,S/AS01$_E$ safety after vaccination as well as vaccine effectiveness and impact. EPI-MAL-003 started when RTS,S/AS01$_E$ was authorized in the country and implemented through an Expanded Programme on Immunization schedule that builds on the routine national immunization program, under the supervision of the Ministry of Health. In parallel with both the EPI-MAL-002 and EPI-MAL-003 studies, a third study, EPI-MAL-005, was conducted to measure malaria transmission intensity and the impact of vaccination on other malaria control interventions such as residual spraying and bed nets on a yearly basis during the rainy season. Study EPI-MAL-005 started in Q4 2014, at the end of the rainy season in the West African sites with peak malaria transmission in the second half of the year, and in Q2 2015 in the East African sites with peak malaria transmission in the first half of the year. It will run until the completion of the EPI-MAL-003 study.

P. falciparum is a pathogen with high genetic variability and a high number of different circulating strains. The parasite has evolved mechanisms to vary cell surface antigens and elude the host's immune response. For this reason, there is a safety concern that RTS,S/AS01$_E$ vaccine selects specific parasite variants or alters the number of parasite haplotypes by exerting a selective pressure over time.

This ancillary study to EPI-MAL-005, referred to as the EPI-MAL-010 study, monitored the genetic diversity in CS sequences in the *P. falciparum* parasite population before and after vaccine implementation in children aged 6 months to <5 years.

6. RESEARCH QUESTION AND OBJECTIVE(S)

Co-primary objectives:

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in participants aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in participants aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.

These co-primary objectives involved pharmacogenomics testing.

Secondary objectives:

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in participants aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in participants aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To estimate trends in longitudinal prevalence of *P. falciparum* in participants aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To estimate trends in longitudinal frequency of *P. falciparum* in participants aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.

These secondary objectives involved pharmacogenomics testing.

7. RESEARCH METHODS

7.1. Study Design

EPI-MAL-010 was a longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study. In order to characterize *P. falciparum* haplotypes, genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, 1 site in Eastern Africa and 1 site in Western Africa. Those sites were the same during the entire duration of the study.

This design allowed monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after the start of RTS,S/AS01$_E$ vaccination, across 7 consecutive annual surveys as described below:

- 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

While reporting the results, the survey numbers were updated as Surveys 3 to 9 to follow exactly the numbering of the EPI-MAL-005 study to facilitate cross-referencing between both studies.

Primary completion date was defined as the date of final collection of data for all primary outcomes, while end of study was defined as the date when last testing results of samples re-used from EPI-MAL-005 were released. End of study was achieved within 8 months after the selection of the samples for testing of the last survey, as per protocol. The study was concluded when results of all the samples from participants enrolled in Survey 9 at Year 7 were received from HSPH/BI after sequencing.

7.2. Study Population/Subjects and Setting

In the EPI-MAL-005 study, a blood sample was obtained for malaria blood slide reading conducted locally, and 2 to 3 drops of blood were spotted onto filter paper for the NAAT (conducted at AMC, Amsterdam, The Netherlands). Both blood slide reading (by microscopy) and NAAT (from genomic DNA) were used to evaluate the level of asexual *P. falciparum* parasitemia. For samples that were identified as positive for *P. falciparum* by malaria blood slide reading and/or by NAAT, a minimum of 15 microliters of DNA extracted at AMC were sent to HSPH/BI for amplicon sequencing. DNA samples collected and processed during the EPI-MAL-005 study were reused for the EPI-MAL-010 study.

In the framework of the MVIP, the RTS,S/AS01$_E$ vaccine was planned to be administered by the Ministry of Health through the Expanded Programme on Immunization as a three-dose primary series, followed by a fourth booster dose. Based on this information and on the current assumption that the first three doses of RTS,S/AS01$_E$ would be administered to all participants within a period of 18 months, conducting the EPI-MAL-010 study during 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study, pre- and post-RTS,S/AS01$_E$ MVIP start, allowed monitoring haplotype diversity in a representative proportion of participants having received the vaccine. Selection of surveys included in EPI-MAL-010 study was contingent upon the start date of the MVIP and required the first surveys to occur prior to MVIP start.

Participants

For details of the study population, refer to Protocol Section 9.2.1. For details regarding sample selection, refer to Protocol Section 9.2.2.

For details of the study period, refer to Protocol Section 9.2.5.

Details of the case definitions are provided in Protocol Section 9.2.6.

Refer to Protocol Section 9.2.7 for details of the study procedures and Section 9.2.8 for biological sample handling and analysis.

Eligibility Criteria

In the MVIP, 3 countries (Ghana, Kenya, and Malawi) were selected for the vaccine implementation, hence EPI-MAL-003 study was conducted in the above mentioned countries. In 2 of these countries (Ghana and Kenya), sites were selected for participation in the EPI-MAL-005 study. Sites from Ghana and Kenya were moderate-to-high transmission areas. Based on the results from MALARIA- 066, selecting 1 site situated in

Western Africa and the other in Eastern Africa allowed further exploration of the previously observed *P. falciparum* strain diversity differences between Western and Eastern Africa, and increased the continental diversity of study participants in EPI-MAL-010.

Inclusion Criteria

Deviations from inclusion criteria were not allowed because they could have potentially jeopardized the scientific integrity and regulatory acceptability of the study or participant safety. Therefore, adherence to the criteria as specified in the protocol was essential.

All participants were required to satisfy ALL the following criteria at study entry:

- Participants aged 6 months to <5 years enrolled in the EPI-MAL-005 study at 2 sites (1 site in Eastern Africa and 1 site in Western Africa), fulfilling inclusion and exclusion criteria of the EPI-MAL-005 study.
- Participants whose parent(s)/LAR(s) had provided IC for the use of collected blood samples in further research as explained in the original ICF of the EPI-MAL-005 study.

Exclusion Criteria

Deviations from exclusion criteria were not allowed because they could have potentially jeopardized the scientific integrity and regulatory acceptability of the study. Therefore, adherence to the criteria as specified in the protocol was essential.

The following criterion were checked at the time of study entry. If ANY exclusion criterion applied, the participant was not included in the study:

All eligible participants from the EPI-MAL-005 study that were going to be 5 years of age or over at time of the annual survey (i.e., at the time of sample collection) were excluded.

7.3. Variables

Co-primary endpoints:

- Occurrence of specific *P. falciparum* haplotype infection at participant/haplotype level.
  - Criteria/definitions: infection with (a) particular *P. falciparum* haplotype(s) confirmed for the CS C-terminus and/or SERA2 loci using sequencing techniques. Based on recommendation by the testing laboratory, infection was confirmed using CS C-terminus for sequencing.

Secondary endpoints

- Occurrence of specific *P. falciparum* haplotype infection at participant level by sub-groups.
- Occurrence of specific *P. falciparum* haplotype infection at haplotypes level by sub-groups.
- Estimate trends of specific *P. falciparum* haplotype infection at participant level by RTS,S/AS01$_E$ vaccination status.
- Estimate trends of specific *P. falciparum* haplotype infection at haplotype level by RTS,S/AS01$_E$ vaccination status.

For trends analyses, only the 3D7 haplotype and the haplotypes with more than 5% of frequency across the 7 surveys were considered.

Primary estimand

The primary question of interest is: Could we characterise *P. falciparum* haplotypes detected in young children aged 6 months to <5 years over time before and after the introduction of the RTS,S/AS01$_E$ vaccine on one site in Eastern Africa and one site in Western Africa, based on samples collected in the framework of the EPI-MAL-005 study?

The estimand is described by the following attributes:

- *Population:* children aged 6 months to <5 years.
- *Biological samples:* re-use DNA eluates collected and processed during the EPI-MAL-005 study.
- *Variable / endpoint:* frequency and prevalence of 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, the 10 most frequent haplotypes detected were presented.

7.4. Data sources

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study.

In order to determine *P. falciparum* haplotypes, genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination, 1 site in Eastern Africa and 1 site in Western Africa.

Moreover, information regarding demographic data, vaccinations status, and blood sampling were obtained using an extraction from the EPI-MAL-005 database for all the participants whose samples were selected for the EPI-MAL-010 study.

7.5. Bias

Bias is possible due to variety of factors (e.g., low number of samples with sequencing results, limited vaccine coverage).

7.6. Study size

See 'Participants and study size' in Section 2 'Synopsis' of this report.

The sample size was per design, limited to the number of participants included in the EPI-MAL-005 study. All eligible children below 5 years of age enrolled in EPI-MAL-005 during 7 cross-sectional surveys may have been included in EPI-MAL-010 regardless of their parasitemia status. Hence, 400 children below 5 years of age were expected to be enrolled every year in each of the two EPI-MAL-010 study sites.

Malaria prevalence was estimated at 25% for children aged 6 months to 5 years in the selected sites based on GSK sponsored studies in the same centers (data from the EPI-MAL-005 study had its first survey collected in 2014-2015).

Depending on the variations in parasite prevalence over the years, around 100 participants were expected to have a positive malaria parasitemia per site and per year.

Also refer to Protocol Section 9.5 for details of determination of study size.

7.7. Data analysis

The statistical analyses were performed using the Statistical Analysis System version 9.4.

Methodology for this final analysis are specified in detail in the SAP Amendment 1 dated 10 December 2024; changes to analyses as specified in the SAP Amendment 1 are detailed in Section 7.7.3.3.

7.7.1. Datasets for analyses

Analysis Set	Definition / Criteria	Analyses Evaluated
AS	Study participants: • aged **6 months to <5 years** enrolled in the EPI-MAL-005 study at **Kintampo** in Ghana (Western Africa) or **Kombewa** in Kenya (Eastern Africa), **fulfilling inclusion and exclusion criteria** of the EPI-MAL-005 study. • whose parent(s)/[LAR(s)] have provided **IC** for the use of collected blood samples and data in **further research** as explained in the original ICF of the EPI-MAL-005 study. • recruited from Survey 3 to Survey 9.	• Study Population

AS=Analysis set; IC=Informed consent; ICF=Informed consent form; LAR=Legally authorized representative

Refer to SAP Amendment 1 Section 4.1.2 for study groups used for statistical analyses.

7.7.2. Interim Analysis

The numbering of surveys starts from Survey 1 to 7 in the protocol. However, this was updated as Surveys 3 to 9 to be aligned with the numbering of the EPI-MAL-005 study, to facilitate the cross-referencing between both studies. An interim analysis was performed using data from Surveys 3, 4, and 5, conducted before vaccination

implementation and Surveys 6 and 7, conducted after vaccination implementation. All objectives except for trends analysis were assessed in the interim analysis. The interim analysis was descriptive.

As the analyses were based on survey year and sites at the time of the interim analysis, haplotype prevalence and haplotype frequency objectives on Surveys 3 to 7 from the EPI-MAL-005 study were computed. Results for Surveys 3 through 7 were described in the IR dated 26 November 2024 (TMF-19489436, Version 2.0).

7.7.3. Primary analysis

7.7.3.1. Main Analytical approach

All primary analyses were reported by site and survey.

The analyses were performed on the 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, the 10 most frequently detected haplotypes were presented.

All CIs were two-sided 95% CIs computed using the exact method [Clopper and Pearson, 1934].

- Occurrence of specific *P. falciparum* haplotype infection at participant level.

The *haplotype prevalence* was estimated as the number of participants infected with a specific *P. falciparum* haplotype, divided by the total number of participants.

- Occurrence of specific *P. falciparum* haplotype infection at haplotype level.

The *haplotype frequency* was estimated as the number of occurrences of a specific *P. falciparum* haplotype, divided by the total number of haplotypes detected in the study population.

Refer to SAP Section 6.1 and 4.2 for analysis of demographics and analysis of co-primary objectives, respectively.

7.7.3.2. Data handling conventions/data transformations

Age in the study was computed in years as the difference between the IC date and the date of birth divided by 365.25 or taken directly as reported in the Case Report Form if the date of birth is unavailable. The age was stratified into the following groups: 0.5 to <2 years, 2 to <5 years.

Refer to SAP Section 4.1.4.5 for details on the handling of missing data.

7.7.3.3. Conduct of analyses

A summary of changes in analyses from the original statistical plan outlined in the protocol is provided in Table 7.1.

Table 7.1 Changes to Protocol Defined Analysis Plan

Protocol Defined Analysis	Statistical Analysis Plan (SAP)-Defined Analysis	Rationale for Changes
• The numbering of surveys starts from Survey 1 to 7 in the protocol.	• We updated the numbering to Survey 3 to Survey 9 to be aligned with the numbering of the EPI-MAL-005 study.	• We updated the survey number to follow exactly the numbering of the EPI-MAL-005 study to facilitate cross-referencing between both studies.
• Clones	• The term “clones”, often used in protocol, was replaced in the SAP by the term “haplotypes”.	• We used haplotypes instead of clones because we did not receive information about details of clones in the laboratory data and in our study. Haplotype is the most relevant analysis level for the study objectives.
• The analyses were planned on all haplotypes in the protocol.	• In the SAP, we restricted the analyses to the 3D7 strain and the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.	• Due to the large number of haplotypes that could be detected, we decided to focus only on most frequent haplotypes.
• Contrast analysis planned to evaluate trends	• We replaced the contrast analysis by producing graphs on prevalence/frequency over survey time.	• The contrast analysis implied too many possible choices of contrast to be tested, as there was no expectation of trends (linear, cubic, quadratic).
• A multinomial logistic regression to estimate trends on haplotype frequency	• We replaced the multinomial logistic regression by producing graphs on prevalence/frequency over survey time.	• A multinomial logistic regression was not applicable in our frequency trends analysis. There were many distinct haplotypes detected in the population. Therefore, it was not possible to evaluate which ones could be correlated. In addition, we had in our study a maximum of 7 points to be analyzed (7 surveys) with the possibility that a haplotype will not be detected during 1 of the surveys causing it to be removed from the model. In conclusion, multinomial logistic regression was not appropriate.
• Analysis planned on threshold of 15 reads	• An additional sensitivity analysis was performed on a threshold of 50 reads	• Due to more sterile samples, the threshold was reassessed and an additional threshold of 50 reads was analyzed.

Protocol Defined Analysis	Statistical Analysis Plan (SAP)-Defined Analysis	Rationale for Changes
	• Addition of piecewise logistic regression models for longitudinal haplotype prevalence analysis.	• We proposed to consider a prespecified breakpoint based on the year following vaccine introduction (Survey 7) and additionally an analysis allowing the model itself to identify a breakpoint. The goal of this approach was to provide a more flexible model that could capture the underlying patterns in the data
Multiple infection Children infected with >1 haplotype	Multiple infection Children who presented a co-infection on several haplotypes	• The variable co-infection used for the multivariable models was re-labelled by multiple infection for more clarity

The original SAP dated 18 January 2024, Section 4.2.3.1 included a "best-case scenario" analysis (refer original SAP Section 4.2.3.1 for details). In this analysis, non-evaluable samples should be considered as negative for the haplotype being studied using the sequencing method (participants with non-evaluable *P. falciparum* haplotypes).

Upon receiving the data for the interim analysis, a substantial number of haplotypes were identified and a significant proportion of missing data was observed.

The decision was made to modify the proposed analyses by incorporating the best-case scenario approach for prevalence estimate into the main analysis. Given the extensive variety of haplotypes observed, it was considered more practical to assume that the non-evaluable samples were negative for a specific haplotype, aligning with the best-case scenario analysis. Additionally, this approach allowed us to maintain a consistent denominator for all prevalence analyses (both main and sensitivity), enabling more accurate comparisons.

The main analysis was planned to exclude non-evaluable samples from the calculation of prevalence estimates (both in the numerator and denominator). By doing so, the main analysis incorporates the best-case scenario principle in the sensitivity analysis. Therefore, the separate best-case scenario analysis can be omitted. In the worst-case scenario analysis, all non-evaluable samples are considered positive for the haplotype in question. By comparing the main analysis and the worst-case scenario, we can determine that the true value of prevalence estimate should fall between the two results. The final analysis results presented in this report followed the same approach, presented below.

7.7.3.4. Sensitivity analyses

7.7.3.4.1. Worst-case scenario

In the context of non-evaluable samples with the sequencing method, the sensitivity analysis considered the non-evaluable samples as positive samples for the haplotype considered. The haplotype prevalence was estimated on the 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, the 10 most frequently detected haplotypes were presented.

7.7.3.4.2. Threshold of 50 reads

The second sensitivity analysis considered a 50 reads threshold for positivity. The haplotype prevalence and frequency were estimated on the 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, the 10 most frequently detected haplotypes were presented.

Refer to SAP Section 4.2.3 for detailed description of the sensitivity analyses.

Initial haplotype threshold of 15 reads was defined in the protocol based on MALARIA-066 study where positive samples were rich with parasite genetic materials. However, based on conclusions of previous studies (MALARIA-066 and MALARIA-095) and internal scientific re-assessment, additional 50 reads threshold was considered due to more sterile samples (less parasite genetic material) in the EPI-MAL-010 study. Therefore, sensitivity analysis was computed on a threshold of 50 reads.

7.7.4. Secondary analysis

As secondary analyses, prevalence and frequency were reported by site and per survey.

The analyses were performed on the 3D7 haplotype (strain included in the RTS,S/AS01$_E$ vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of <10 haplotypes with a frequency ≥5%, the 10 most frequently detected haplotypes were presented.

7.7.4.1. Prevalence and frequency on sub-groups

The final analysis included a table regrouping all survey data (each haplotype reported within at least one survey) by site and by Survey.

All CIs were two-sided 95% CIs computed using the exact method [Clopper and Pearson, 1934].

For occurrence of specific *P. falciparum* haplotype infection at participant level by sub-groups, the haplotype prevalence was estimated by gender, age group and RTS,S/AS01$_E$ vaccination status. Please see SAP Section 5.1.2 for power considerations for prevalence analyses.

For occurrence of specific *P. falciparum* haplotype infection at haplotypes level by sub-groups, the haplotype frequency (refer SAP Section 4.1.4.2) was estimated by gender, age group and RTS,S/AS01$_E$ vaccination status. Please see SAP Section 5.1.3 for power considerations for frequency analyses.

7.7.4.2. Trend analysis

The trends analysis was done only on the 3D7 haplotype and the haplotypes with more than 5% of frequency across the 7 surveys and reported by site according to RTS,S/AS01$_E$ vaccination status.

- For estimation of trends of specific *P. falciparum* haplotype infection at participant level by RTS,S/AS01$_E$ vaccination status, trends and annual fluctuations in haplotype prevalence were analyzed using logistic regression corresponding to crude and adjusted OR. This method allowed studying trends without making assumptions regarding annual fluctuations (See Table 7.1).
- No adjustment for multiple testing were employed. The assumptions of the logistic regression models were verified (multicollinearity, linearity). Logistic models were used to describe the nature of the relationship between the dependent variable (probability of having *P. falciparum* infection with a given haplotype) and the survey's year. In addition, piecewise logistic regression models were used to describe the nature of the relationship between the dependent variable (specific haplotype infection at participant level) and the survey's year. A prespecified breakpoint based on the year following vaccine introduction (Survey 7) and an additional analysis allowing the model itself to identify a breakpoint was employed. The goal of this approach was to provide a more flexible model that captured the underlying patterns in the data.
- Crude OR and 95% CI were estimated using a univariable logistic regression.
- Adjusted OR and 95% CI were estimated using a multivariable logistic regression model adjusted on gender, age groups, vaccination status and presence/absence of co-infection.
- For estimating trends of specific *P. falciparum* haplotype infection at haplotype level by RTS,S/AS01$_E$ vaccination status, the haplotypes frequencies on the 3D7 haplotype and the haplotypes with >5% of frequency across the 7 surveys were presented over survey time points with bar charts according to RTS vaccination status.

Line plot graphs were generated to present the haplotype prevalence of the 3D7 haplotype and the haplotypes with >5% of frequency across the 7 surveys over survey time points. All graphs were generated site-wise.

7.7.5. Amendments to statistical plan

The SAP was amended once. The SAP Amendment 1 dated 10 December 2024 is provided in the Appendix to this report.

7.8. Quality control and Quality Assurance

Refer to Protocol Section 9.10 for details of quality control measures taken during the conduct of the study.

To ensure compliance with Good Clinical Laboratory Practices and other applicable guidelines and all applicable regulatory requirements, BI was subject to audit in October 2022 by GSK's R&D GQC –CQA department.

The AMC center was subject to audit in July 2023 by GSK's R&D GQC –CQA department.

Up to the data lock point of the interim analysis, 4 significant quality issues were reported from audit conducted in BI and AMC. These issues were investigated and where possible corrective and / or preventive actions were taken. Details are described in the IR dated 26 November 2024.

8. PROTECTION OF HUMAN SUBJECTS

8.1. Ethical approval and subject consent

This study complied with all applicable laws regarding participant privacy. No direct participant contact or primary collection of individual human participant data occurred. Study results are in tabular form and presented as aggregate analyses that omit participant identification, therefore IC was not required. None of the publications and reports include participant identifiers.

The EPI-MAL-010 study was conducted in accordance with all applicable regulatory requirements, including all applicable participant privacy requirements. Conduct of the study included institutional review board/independent ethics committee review and approval of study protocol and any subsequent amendments.

The EPI-MAL-010 study re-used samples collected during the EPI-MAL-005 study. The ICF that was signed during the enrolment for the EPI-MAL-005 study was checked to make sure that the parent(s)/LAR(s) gave their consent for further research on the study sample collected in the EPI-MAL-005 study. For participants whose parent(s)/LAR(s) agreed that their child's/ward's samples may be used for future research, parasite sequencing techniques was performed in the EPI-MAL-010 study using blood samples collected in the EPI-MAL-005 study on samples that tested positive by microscopy or NAAT.

8.2. Participant confidentiality

GSK ensured protection of the personal data of the participants which were collected during the EPI-MAL-005 study.

The following participant confidentiality was maintained for the study:

- Assignment of a unique identifier to each participant by the sponsor. Any participant records or datasets that are transferred to the sponsor contained the identifier only; participants' names or any information which would make the participant identifiable were not transferred.

9. RESULTS

The numbering of surveys starts from Survey 1 to 7 in the protocol. However, this was updated as Survey 3 to Survey 9 to be aligned with the numbering of the EPI-MAL-005 study, to facilitate cross-referencing between both studies. Results for Surveys 3 through 7 were described in the IR dated 26 November 2024 (TMF-19489436, Version 2.0).

Since no new results were obtained between Interim and Final analyses that would alter the results already presented in the IR, Tables 14.1.1.1 to 14.5.6.2/14.1.6.2 were not re-run for the Final analysis and are not presented in this Clinical Study Report.

9.1. Participants

In the EPI-MAL-005 study, freely given and written or thumb-printed IC was obtained from each participant's parent(s)/LAR(s) and the impartial witness as appropriate, prior to participation in the study. For participants whose parent(s)/LAR(s) agreed that their child's/ward's samples may be used for future research, parasite sequencing techniques were performed in the EPI-MAL-010 study using blood samples collected in the EPI-MAL-005 study on samples that tested positive by microscopy or NAAT. There was no additional IC for the EPI-MAL-010 study.

9.1.1. Disposition of participants

Participants in the Analysis set were distributed over 2 centers (Kintampo and Kombewa) in 2 countries (Ghana and Kenya) respectively. The vaccination program was initiated after Survey 5.

Sample validity by site and survey is provided in Table 14.8.2.1. Results for haplotype frequency by site and haplotypes according to survey are provided in Table 14.8.2.2.

The Analysis set (inclusive of both sites in each survey) included 801 participants in Survey 3 (Table 14.1.1.1), 796 participants in Survey 4 (Table 14.2.1.1), 801 participants in Survey 5 (Table 14.3.1.1), 801 participants in Survey 6 (Table 14.4.1.1), 799 participants in Survey 7 (Table 14.5.1.1), 800 participants in Survey 8 (Table 14.6.1.1, presented in Table 9.1) and 801 participants in Survey 9 (Table 14.7.1.1, presented in Table 9.2).

GH Kintampo:

Out of the total number of participants included at each survey in Ghana, the number of participants who were eligible for sample analysis (positive for *P. falciparum* by malaria blood slide reading and/or by NAAT) is provided in Table 14.8.2.1. From the eligible samples, the proportion of participants with valid samples analyzed with cut-off of 15 reads is provided below, with the number of CSP haplotypes detected at each survey:

- Survey 3: 158 samples were eligible and 125 (79.1%) samples were analyzed (Table 14.8.2.1) yielding 340 haplotypes (Table 14.1.1.1)

- Survey 4: 152 samples were eligible and 122 (80.3%) samples were analyzed (Table 14.8.2.1) yielding 294 haplotypes (Table 14.2.1.1)
- Survey 5: 106 samples were eligible and 68 (64.2%) samples were analyzed (Table 14.8.2.1) yielding 121 haplotypes (Table 14.3.1.1)
- Survey 6: 92 samples were eligible and 81 (88.0%) samples were analyzed (Table 14.8.2.1) yielding 219 haplotypes (Table 14.4.1.1)
- Survey 7: 74 samples were eligible and 55 (74.3%) samples were analyzed (Table 14.8.2.1) yielding 85 haplotypes (Table 14.5.1.1)
- Survey 8: 44 samples were eligible and 28 (63.6%) samples were analyzed (Table 14.8.2.1) yielding 64 haplotypes (Table 9.1)
- Survey 9: 49 samples were eligible and 31 (63.3%) samples were analyzed (Table 14.8.2.1) yielding 71 haplotypes (Table 9.2).

3D7 haplotype was not detected during Survey 8 in GH Kintampo.

KE Kombewa:

Out of the total number of participants included at each survey in Kenya, the number of participants who were eligible for sample analysis (positive for *P. falciparum* by malaria blood slide reading and/or by NAAT) is provided in Table 14.8.2.1. From the eligible samples, the proportion of participants with valid samples analyzed with cut-off of 15 reads is provided below, with the number of CSP haplotypes detected at each survey:

- Survey 3: 181 samples were eligible and 145 (80.1%) samples were analyzed (Table 14.8.2.1) yielding 361 haplotypes (Table 14.1.1.1)
- Survey 4: 174 samples were eligible and 140 (80.5%) samples were analyzed (Table 14.8.2.1) yielding 364 haplotypes (Table 14.2.1.1)
- Survey 5: 172 samples were eligible and 127 (73.8%) samples were analyzed (Table 14.8.2.1) yielding 297 haplotypes (Table 14.3.1.1)
- Survey 6: 162 samples were eligible and 128 (79.0%) samples were analyzed (Table 14.8.2.1) yielding 358 haplotypes (Table 14.4.1.1)
- Survey 7: 129 samples were eligible and 102 (79.1%) samples were analyzed (Table 14.8.2.1) yielding 245 haplotypes (Table 14.5.1.1)
- Survey 8: 126 samples were eligible and 92 (73.0%) samples were analyzed (Table 14.8.2.1) yielding 206 haplotypes (Table 9.1)
- Survey 9: 118 samples were eligible and 78 (66.1%) samples were analyzed (Table 14.8.2.1) yielding 168 haplotypes (Table 9.2)

3D7 haplotype was not detected during Survey 7 in KE Kombewa.

9.2. Descriptive data including baseline characteristics

Demographic and baseline characteristics of the participants in Survey 3, Survey 4, Survey 5, Survey 6, and Survey 7 are presented in the IR dated 26 November 2024 (TMF-19489436, Version 2.0).

Demographics and baseline characteristics of participants in Survey 8 and Survey 9 are presented in Table 14.6.1.1 (Table 9.1) and Table 14.7.1.1 (Table 9.2), respectively.

9.2.1. Demographic characteristics

Demographic results for Surveys 3 through 7 are available in detail, in the IR dated 26 November 2024 (TMF-19489436, Version 2.0), while a summary is provided in this report in addition to detailed description of results for Surveys 8 and 9.

Overall demographic characteristics are described below for the 2 sites for Surveys 8 and 9: GH Kintampo and KE Kombewa (Table 9.1 and Table 9.2, respectively).

GH Kintampo: The mean (SD) age at IC for each survey at the site in Ghana was as follows:

- 2.32 (1.192) years in Survey 3 (Table 14.1.1.1)
- 2.33 (1.165) years in Survey 4 (Table 14.2.1.1)
- 2.39 (1.130) years in Survey 5 (Table 14.3.1.1)
- 2.29 (1.164) years in Survey 6 (Table 14.4.1.1)
- 2.31 (1.156) years in Survey 7 (Table 14.5.1.1)
- 2.28 (1.187) years in Survey 8 (Table 9.1), and
- 2.31 (1.175) years in Survey 9 (Table 9.2).

During Surveys 8 and 9 at the site in Ghana, a majority of participants were of annual age 2 years (30.2% in Survey 8 and 30.8% in Survey 9), a higher proportion were within the 2 to 4 years age group (55.3% in Survey 8 and 56.0% in Survey 9), and a majority of the participants were female (53.5% in Survey 8 and 51.5% in Survey 9) (Table 9.1 and Table 9.2).

KE Kombewa: The mean (SD) age at IC for each survey at the site in Kenya was as follows:

- 2.36 (1.184) years in Survey 3 (Table 14.1.1.1)
- 2.34 (1.188) years in Survey 4 (Table 14.2.1.1)
- 2.33 (1.221) years in Survey 5 (Table 14.3.1.1)
- 2.33 (1.183) years in Survey 6 (Table 14.4.1.1)
- 2.40 (1.191) years in Survey 7 (Table 14.5.1.1)
- 2.30 (1.193) years in Survey 8 (Table 9.1), and

- 2.38 (1.192) years in Survey 9 (Table 9.2).

At the site in Kenya, a higher proportion of participants were of annual age 2 years (29.6%) and a majority were female (55.5%) in Survey 8, while a higher proportion of participants were of annual age 1 year (29.9%). Across both Surveys 8 and 9, a higher proportion of participants were within the 2 to 4 years age group (54.5% in Survey 8 and 54.9% in Survey 9) (Table 9.1 and Table 9.2).

Table 9.1 Summary of demographic characteristics by site - Analysis Set, Survey 8

			Total	
Site	Characteristics	Parameters or Categories	Value or n	%
GH_Kintampo	Number of participants	N	398	-
	Age at IC [years]	Mean	2.28	-
		Standard Deviation	1.187	-
		Median	2.23	-
		Minimum	0.6	-
		Maximum	4.9	-
	Annual age	0 years	60	15.1
		1 year	118	29.6
		2 years	120	30.2
		3 years	49	12.3
		4 years	51	12.8
	WHO age group [years]	0.5-1Y	178	44.7
		2-4Y	220	55.3
	Gender	Female	213	53.5
		Male	185	46.5
	RTS,S vaccination status	Unvaccinated	142	35.7
		Vaccinated	256	64.3
KE_Kombewa	Number of participants	N	402	-
	Age at IC [years]	Mean	2.30	-
		Standard Deviation	1.193	-
		Median	2.15	-
		Minimum	0.5	-
		Maximum	5.0	-
	Annual age	0 years	65	16.2
		1 year	118	29.4
		2 years	119	29.6
		3 years	52	12.9
		4 years	48	11.9
	WHO age group [years]	0.5-1Y	183	45.5
		2-4Y	219	54.5
	Gender	Female	223	55.5
		Male	179	44.5
	RTS,S vaccination status	Unvaccinated	163	40.5
		Vaccinated	239	59.5

GH = Ghana; IC = Informed consent; KE = Kenya; N = total number of eligible participants; n = number of participants in a given category; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins/ GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; WHO = World Health Organisation.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date
% = n/N × 100
Value = value of the considered parameter

Source: Table 14.6.1.1 (08APR2025 23:41 GMT)

Table 9.2 Summary of demographic characteristics by site - Analysis Set, Survey 9

			Total	
Site	Characteristics	Parameters or Categories	Value or n	%
GH_Kintampo	Number of participants	N	400	-
	Age at IC [years]	Mean	2.31	-
		Standard Deviation	1.175	-
		Median	2.26	-
		Minimum	0.5	-
		Maximum	4.9	-
	Annual age	0 years	59	14.8
		1 year	117	29.3
		2 years	123	30.8
		3 years	52	13.0
		4 years	49	12.3
	WHO age group [years]	0.5-1Y	176	44.0
		2-4Y	224	56.0
	Gender	Female	206	51.5
		Male	194	48.5
	RTS,S vaccination status	Unvaccinated	100	25.0
		Vaccinated	300	75.0
KE_Kombewa	Number of participants	N	401	-
	Age at IC [years]	Mean	2.38	-
		Standard Deviation	1.192	-
		Median	2.27	-
		Minimum	0.5	-
		Maximum	5.0	-
	Annual age	0 years	61	15.2
		1 year	120	29.9
		2 years	119	29.7
		3 years	50	12.5
		4 years	51	12.7
	WHO age group [years]	0.5-1Y	181	45.1
		2-4Y	220	54.9
	Gender	Female	199	49.6
		Male	202	50.4
	RTS,S vaccination status	Unvaccinated	96	23.9
		Vaccinated	305	76.1

GH = Ghana; IC = Informed consent; KE = Kenya; N = total number of eligible participants; n = number of participants in a given category; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; WHO = World Health Organisation.

WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date

% = n/N × 100

Value = value of the considered parameter

Source: Table 14.7.1.1 (08APR2025 23:41 GMT)

9.2.2. Vaccination history of RTS,S/AS01$_E$

Surveys 3 and 4 were conducted and Survey 5 was initiated before the start of RTS,S/AS01$_E$ vaccination, while Surveys 6, 7, 8, and 9 were conducted after the start of RTS,S/AS01$_E$ vaccination.

Details regarding Survey 6 and Survey 7 are presented in the IR dated 26 November 2024. Vaccination history by age group is described below for Survey 8 (Table 14.6.1.2, presented in Table 9.3) and Survey 9 (Table 14.7.1.2, presented in Table 9.4) for each site.

GH Kintampo:

Out of the 398 participants from GH Kintampo during Survey 8, overall 64.3% participants were vaccinated, i.e., received at least 1 dose (Table 9.1), where the proportion of vaccinated participants was 87.1% in the 0.5 to 1 year age group and 45.9% in the 2 to 4 years age group (Table 9.3). The majority of participants in the 0.5 to 1 year age group had received 3 doses (64.0%), while the majority in the 2 to 4 years age group had not been vaccinated (54.1%). For the youngest children this is the period to receive vaccination so potentially some children are too young to receive the third dose.

Out of the 400 participants from GH Kintampo during Survey 9, overall 75.0% participants were vaccinated, i.e., received at least 1 dose (Table 9.2), where the proportion of vaccinated participants was 84.7% in 0.5 to 1 year age group and 67.4% in 2 to 4 years age group (Table 9.4). The majority of participants in the 0.5 to 1 year age group had received 3 doses (57.4%), 36.2% in the 2 to 4 years age group had received 4 doses while 32.6% had not been vaccinated. No participant in the 0.5 to 1 year age group had received the 4th dose, while the mean age (in months) of participants in the 2 to 4 years age group at the 4th dose was 25.46 months (SD=3.368; n=81). In GH Kintampo, overall, 1 participant in the 0.5 to 1 year age group received the 4th dose during Survey 8, while 148 participants in the 2 to 4 years age group received the 4th dose across Surveys 7 to 9.

KE Kombewa:

Out of the 402 participants from KE Kombewa during Survey 8, overall 59.5% participants were vaccinated, i.e., received at least 1 dose (Table 9.1), where the proportion of vaccinated participants was 72.7% in the 0.5 to 1 year age group and 48.4% in the 2 to 4 years age group (Table 9.3). The majority of participants in the 0.5 to 1 year age group had received 3 doses (57.9%), while the majority in the 2 to 4 years age group had not been vaccinated (51.6%) and a lower proportion (28.3%) in the 2 to 4 years age group had received 4 doses.

Out of the 401 participants from KE Kombewa during Survey 9, overall 76.1% participants were vaccinated, i.e., received at least 1 dose (Table 9.2), where the proportions of vaccinated participants were: 91.2% (0.5 to 1 year age group) and 63.6% (2 to 4 years age group), see Table 9.4 for details. The majority of participants in the 0.5 to 1 year age group had received 3 doses (74.6%), 36.4% in the 2 to 4 years age group had not been vaccinated and 31.8% in the 2 to 4 years age group had received 4 doses.

No participant in the 0.5 to 1 year age group had received the 4th dose, while the mean age (in months) of participants in the 2 to 4 years age group at the 4th dose was 24.48 months (SD=1.905; n=70). In KE Kombewa, overall, 3 participants in the 0.5 to 1 year age group received the 4th dose during Surveys 7 and 8, while 140 participants in the 2 to 4 years age group received the 4th dose across Surveys 7 to 9.

Table 9.3 Vaccination history of RTS,S/AS01$_E$ by site and according to age group - Analysis Set, Survey 8

			0.5-1Y		2-4Y	
Site	**Characteristics**	**Parameters or Categories**	**Value or n**	**%**	**Value or n**	**%**
GH_Kintampo	RTS,S vaccination status	Unvaccinated	23	12.9	119	54.1
		Vaccinated	155	87.1	101	45.9
	Number of doses of RTS,S	0 doses	23	12.9	119	54.1
		1 dose	14	7.9	1	0.5
		2 doses	26	14.6	2	0.9
		3 doses	114	64.0	32	14.5
		4 doses	1	0.6	66	30.0
	Age in months at RTS,S vaccination [1st dose]	n	155	-	101	-
		Mean	6.90	-	7.32	-
		Standard Deviation	1.256	-	2.942	-
		Median	6.64	-	6.54	-
		Minimum	4.4	-	5.2	-
		Maximum	13.7	-	29.2	-
	Age in months at RTS,S vaccination [2nd dose]	n	141	-	100	-
		Mean	8.36	-	8.82	-
		Standard Deviation	1.594	-	3.558	-
		Median	7.89	-	7.75	-
		Minimum	5.5	-	6.4	-
		Maximum	16.5	-	30.2	-
	Age in months at RTS,S vaccination [3rd dose]	n	115	-	98	-
		Mean	10.51	-	10.94	-
		Standard Deviation	1.931	-	3.478	-
		Median	9.89	-	9.71	-
		Minimum	7.5	-	8.3	-
		Maximum	18.8	-	31.2	-
	Age in months at RTS,S vaccination [4th dose]	n	1	-	66	-
		Mean	13.80	-	24.72	-
		Standard Deviation		-	3.395	-
		Median	13.80	-	24.72	-
		Minimum	13.8	-	11.8	-

			0.5-1Y		2-4Y	
Site	**Characteristics**	**Parameters or Categories**	**Value or n**	**%**	**Value or n**	**%**
		Maximum	13.8	-	37.1	-
KE_Kombewa	RTS,S vaccination status	Unvaccinated	50	27.3	113	51.6
		Vaccinated	133	72.7	106	48.4
	Number of doses of RTS,S	0 doses	50	27.3	113	51.6
		1 dose	5	2.7	5	2.3
		2 doses	21	11.5	9	4.1
		3 doses	106	57.9	30	13.7
		4 doses	1	0.5	62	28.3
	Age in months at RTS,S vaccination [1st dose]	n	133	-	106	-
		Mean	6.22	-	6.82	-
		Standard Deviation	0.593	-	1.354	-
		Median	6.14	-	6.29	-
		Minimum	3.5	-	5.2	-
		Maximum	9.1	-	11.7	-
	Age in months at RTS,S vaccination [2nd dose]	n	128	-	101	-
		Mean	7.40	-	8.08	-
		Standard Deviation	0.717	-	1.556	-
		Median	7.20	-	7.43	-
		Minimum	6.0	-	6.3	-
		Maximum	11.5	-	15.0	-
	Age in months at RTS,S vaccination [3rd dose]	n	107	-	92	-
		Mean	9.40	-	10.55	-
		Standard Deviation	0.797	-	3.333	-
		Median	9.20	-	9.35	-
		Minimum	8.1	-	8.2	-
		Maximum	15.3	-	25.2	-
	Age in months at RTS,S vaccination [4th dose]	n	1	-	62	-
		Mean	12.35	-	24.24	-
		Standard Deviation		-	3.661	-
		Median	12.35	-	24.28	-
		Minimum	12.4	-	12.0	-
		Maximum	12.4	-	36.0	-

GH = Ghana; IC=Informed consent; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date
n/% = number / percentage of participants in a given category
Value = value of the considered parameter
Source: Table 14.6.1.2 (08APR2025 23:41 GMT)

Table 9.4 Vaccination history of RTS,S/AS01$_E$ by site and according to age group - Analysis Set, Survey 9

			0.5-1Y		2-4Y	
Site	Characteristics	Parameters or Categories	Value or n	%	Value or n	%
GH_Kintampo	RTS,S vaccination status	Unvaccinated	27	15.3	73	32.6
		Vaccinated	149	84.7	151	67.4
	Number of doses of RTS,S	0 doses	27	15.3	73	32.6
		1 dose	19	10.8	4	1.8
		2 doses	29	16.5	3	1.3
		3 doses	101	57.4	63	28.1
		4 doses	0	0	81	36.2
	Age in months at RTS,S vaccination [1st dose]	n	149	-	151	-
		Mean	6.82	-	7.14	-
		Standard Deviation	1.385	-	2.537	-
		Median	6.57	-	6.64	-
		Minimum	1.5	-	3.9	-
		Maximum	13.8	-	32.4	-
	Age in months at RTS,S vaccination [2nd dose]	n	130	-	147	-
		Mean	8.49	-	8.55	-
		Standard Deviation	1.759	-	2.690	-
		Median	7.98	-	7.89	-
		Minimum	5.2	-	5.6	-
		Maximum	17.6	-	33.3	-
	Age in months at RTS,S vaccination [3rd dose]	n	101	-	144	-
		Mean	10.77	-	10.96	-
		Standard Deviation	2.129	-	3.087	-
		Median	9.99	-	10.00	-
		Minimum	7.5	-	7.5	-
		Maximum	18.6	-	34.3	-
	Age in months at RTS,S vaccination [4th dose]	n	0	-	81	-
		Mean		-	25.46	-
		Standard Deviation		-	3.368	-
		Median		-	24.74	-
		Minimum		-	12.6	-
		Maximum		-	38.2	-
KE_Kombewa	RTS,S vaccination status	Unvaccinated	16	8.8	80	36.4
		Vaccinated	165	91.2	140	63.6
	Number of doses of RTS,S	0 doses	16	8.8	80	36.4
		1 dose	10	5.5	3	1.4
		2 doses	20	11.0	9	4.1
		3 doses	135	74.6	58	26.4
		4 doses	0	0	70	31.8

			0.5-1Y		2-4Y	
Site	**Characteristics**	**Parameters or Categories**	**Value or n**	**%**	**Value or n**	**%**
	Age in months at RTS,S vaccination [1st dose]	n	165	-	140	-
		Mean	6.23	-	6.68	-
		Standard Deviation	0.654	-	1.469	-
		Median	6.11	-	6.18	-
		Minimum	1.1	-	5.4	-
		Maximum	9.0	-	14.7	-
	Age in months at RTS,S vaccination [2nd dose]	n	155	-	137	-
		Mean	7.47	-	7.87	-
		Standard Deviation	0.826	-	2.006	-
		Median	7.16	-	7.26	-
		Minimum	6.4	-	6.2	-
		Maximum	11.5	-	24.0	-
	Age in months at RTS,S vaccination [3rd dose]	n	135	-	128	-
		Mean	9.38	-	9.91	-
		Standard Deviation	0.816	-	2.138	-
		Median	9.13	-	9.17	-
		Minimum	8.0	-	8.0	-
		Maximum	14.1	-	23.9	-
	Age in months at RTS,S vaccination [4th dose]	n	0	-	70	-
		Mean	-	-	24.48	-
		Standard Deviation	-	-	1.905	-
		Median	-	-	24.21	-
		Minimum	-	-	14.1	-
		Maximum	-	-	30.8	-

GH = Ghana; IC=Informed consent; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date
n/% = number / percentage of participants in a given category
Value = value of the considered parameter
Source: Table 14.7.1.2 (08APR2025 23:41 GMT)

9.3. Results of co-primary analyses

All haplotypes detected during this study are provided in a listing of full nucleotide sequences with CIGAR string associated with the CSP amplicon, in Listing 1.1.

To improve readability, each unique haplotype's CIGAR string presented in this report is represented by a short code. The key for these codes is presented in Table 9.5 below.

Table 9.5 CIGAR strings reported during Surveys

CIGAR strings	Country	ID's (CIGXY*)
298K301N317E318Q321K322T352G354S356N361E"'	Ghana	CIG11
301N317E318Q321K322R352G356E359N361E"'	Ghana	CIG110
294N301N317E318K321K322E357Q"'	Ghana	CIG111
295K301N317E321K324K327I357Q359N361E"'	Ghana	CIG112
301N314Q317E318K321K"'	Ghana	CIG113
301N302N317E318Q321K322T357Q361E"'	Ghana	CIG114
301N302N317E318Q321K357Q361E"'	Ghana	CIG115
301N302N317E318Q321K357Q"'	Ghana	CIG116
301N302N317E318Q321K361E"'	Ghana	CIG117
301D314Q317E318K321K322I324K357Q"'	Ghana	CIG118
301N317E318Q321K322R357Q"'	Ghana	CIG119
301N317E318Q321K322T324K'	Ghana	CIG12
301N314Q317E318K321K322T"'	Ghana	CIG120
301N321K322I352G356E359N361E"'	Ghana	CIG13
301N314Q317E318K321K357Q"'	Ghana	CIG14
298K301N317E318Q321K322T361E"'	Ghana	CIG15
298K301N317E318Q321K361E"'	Ghana	CIG16
301N302G317E318Q321K352G354S361E'	Ghana	CIG17
301N317E318Q321K322R352D357Q"'	Ghana	CIG18
301N317E318Q321K322R352G"'	Ghana	CIG19
301N314Q317E318K321Q324K357Q359N361E"	Ghana	CIG121
301N317E318Q321K322T324K359N361E"	Ghana	CIG122
296D301N317E318Q321K322T324K357Q"	Ghana	CIG123
301N314Q317E318K324K349D357Q"	Ghana	CIG124
298K301N317E318Q321K322T324K357Q361E"	Ghana	CIG125
301N317E318Q324K327I359N361E'	Kenya	CIG21
301N327I352D357Q361I'	Kenya	CIG22
298K301N327I352D357Q361I"'	Kenya	CIG23
301N317T321K352G354S356N361E'	Kenya	CIG24
301N317E318Q324K327I361E"'	Kenya	CIG25
301N317T321K322R352G354S356N361E'	Kenya	CIG26
301N317E318Q321K322T324K357Q"'	Kenya	CIG27
301N314Q317E318K321K322R352D357Q"	Kenya	CIG28
298K301N317E318Q321K322T357Q'	Ghana and Kenya	CIG31
301N314Q317E318K321Q357Q359N361E'	Ghana and Kenya	CIG310
295K301N317E321K324K327I361E"'	Ghana and Kenya	CIG311
301N314Q317E318K321K322R357Q'	Ghana and Kenya	CIG312
301N314Q317E318K321K322T361E"'	Ghana and Kenya	CIG313
301N314Q317E318K321K322I324K357Q'	Ghana and Kenya	CIG314
294N301N317E318K321K322E352G356N361E'	Ghana and Kenya	CIG315
298K301N317E318Q321K322T357Q361E'	Ghana and Kenya	CIG32
301N314Q317E318K321K322T357Q'	Ghana and Kenya	CIG33
301N314Q317E318K324K357Q'	Ghana and Kenya	CIG34
301N317E318Q321K322T324K357Q361E'	Ghana and Kenya	CIG35
301N317E318Q321K324K327I361E'	Ghana and Kenya	CIG36
301N317T321K324K327I361E'	Ghana and Kenya	CIG37
301N314Q317E318K321K322I357Q'	Ghana and Kenya	CIG38
301N314Q317E318K321K322T324K357Q'	Ghana and Kenya	CIG39
301N314Q317E318K321K322T324K	Ghana	CIG126
301N317E318Q321K322I324K357Q	Ghana	CIG127

CIGAR = Compact Idiosyncratic Gapped Alignment Report.
*: CIGXY: X: the country this string was detected in: 1 = Ghana, 2 = Kenya, 3 = Ghana and Kenya; Y: sequential number for CIGAR string (can be 1 or 2 numerals).
(') : Surveys 3 to 9 (1-7); (''): Surveys 3 to 7 (1-5); ('''): Surveys 8 to 9 (6-7)
Notes: Codes were built based on the CIGAR strings (haplotypes) reported in the interim and final analyses
Only haplotypes with a frequency ≥5% in the site during the survey were reported. In case of <10 haplotypes with a frequency ≥5%, the 10 most frequent haplotypes were reported (per protocol).

9.3.1. Longitudinal Estimates of *P. falciparum* Haplotype Prevalence by site

The prevalence of *P. falciparum* haplotypes by site, i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Survey 3 (Table 14.1.3.1), Survey 4 (Table 14.2.3.1), Survey 5 (Table 14.3.3.1), Survey 6 (Table 14.4.3.1), and Survey 7 (Table 14.5.3.1) is presented in the IR dated 26 November 2024. The prevalence of *P. falciparum* haplotypes, i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Survey 8 and Survey 9 are provided in Table 14.6.3.1 (presented in Table 9.6) and Table 14.7.3.1 (presented in Table 9.7), respectively.

Results for haplotype prevalence by site and haplotypes according to survey are provided in Table 14.8.2.3.

A graphical representation of prevalence of *P. falciparum* haplotypes during Survey 8 is provided in Figure 14.6.3.2 (GH Kintampo) and Figure 14.6.3.3 (KE Kombewa), while prevalence during Survey 9 is provided in Figure 14.7.3.2 (GH Kintampo) and Figure 14.7.3.3 (KE Kombewa).

GH Kintampo:

Survey 3: Among all detected haplotypes, 5 had a prevalence of >5%, individually. They were 3D7, CIG32, CIG33, CIG38, and CIG39. The most prevalent haplotype was CIG39 with a prevalence of 7.23% as compared to 6.23% for 3D7 haplotype (Table 14.1.3.1).

Survey 4: Among all detected haplotypes, 3 had a prevalence of >5%, individually. They were 3D7, CIG32, and CIG39. The most prevalent haplotype was CIG32 with a prevalence of 8.29% as compared to 5.78% for 3D7 haplotype (Table 14.2.3.1).

Survey 5: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 with a prevalence of 2.76% as compared to 2.51% for 3D7 haplotype (Table 14.3.3.1).

Survey 6: Among all detected haplotypes, 1 had a prevalence of >5%: CIG32 and it was the most prevalent haplotype. CIG32 had a prevalence of 5.72% as compared to 2.49% for 3D7 haplotype (Table 14.4.3.1).

Survey 7: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 which had a prevalence of 2.26% as compared to 2.01% for 3D7 haplotype (Table 14.5.3.1).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (3.02%), where 3 haplotypes showed prevalence of >1% and 3D7 was not detected (Table 9.6).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG38 and CIG310 (1.25% prevalence, each), where 3 haplotypes showed prevalence of ≥1% and 3D7 was detected for 1 participant (0.25%) (Table 9.7).

Across all surveys in GH Kintampo, 5 haplotypes had a prevalence of >5%: 3D7 (prevalence of 6.23% during Survey 3, 5.78% during Survey 4), CIG32 (prevalence of 8.29% during Survey 4, 5.49% during Survey 3, 5.72% during Survey 6), CIG33 (5.24% during Survey 3), CIG38 (6.23% during Survey 3), and CIG39 (prevalence of 7.23% during Survey 3, 5.03% during Survey 4). CIG32 was the most prevalent across most surveys (Surveys 4, 5, 6, 7, and 8).

The prevalence of 3D7 haplotype was highest during Survey 3 (6.23%) and showed a gradual decline to Survey 9 (0.25%) and was not detected during Survey 8. Other haplotypes detected in GH Kintampo did not show any clear trend in prevalence across surveys, except for the following (Table 14.8.2.3):

- CIG33 showed a decrease in prevalence from Survey 3 (5.24%) to Survey 5 (1.50%), which fluctuated till Survey 9 (1.00%).
- CIG38 showed a gradual decrease in prevalence from Survey 3 (6.23%) to Survey 8 (0.25%), with a slight increase at Survey 9 (1.25%)
- CIG39 showed a gradual decrease in prevalence from Survey 3 (7.23%) to Survey 8 (0.25%), with a slight increase at Survey 9 (0.75%)

KE Kombewa:

Survey 3: Among all detected haplotypes, 3 had a prevalence of >5%. They were CIG32, CIG33, and CIG38. The most prevalent haplotype was CIG32, which had a prevalence of 9.25% as compared to 1.00% for 3D7 haplotype (Table 14.1.3.1).

Survey 4: Among all detected haplotypes, 5 had a prevalence of >5%. They were CIG32, CIG33, CIG35, CIG38, and CIG39. The most prevalent haplotype was CIG32, which had a prevalence of 11.31% as compared to 0.25% for 3D7 haplotype (Table 14.2.3.1).

Survey 5: Among all detected haplotypes, 4 had a prevalence of >5%. They were CIG32, CIG33, CIG35, and CIG38. The most prevalent haplotype was CIG33 which had a prevalence of 8.46% as compared to 1.00% for 3D7 haplotype (Table 14.3.3.1).

Survey 6: Among all detected haplotypes, 4 had a prevalence of >5%. They were CIG32, CIG33, CIG35, and CIG38. The most prevalent haplotype was CIG32 which had a prevalence of 10.78% as compared to 0.50% for 3D7 haplotype (Table 14.4.3.1).

Survey 7: Among all detected haplotypes, 2 had a prevalence of >5%. They were CIG32 and CIG33. The most prevalent haplotype was CIG33 which had a prevalence of 6.00% as compared to 0% for 3D7 haplotype (Table 14.5.3.1,).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG39 (3.73%), where all 10 haplotypes showed prevalence of >1% and 3D7 was detected in 3 participants (0.75%) (Table 9.6).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG32 and CIG33 (3.74% prevalence, each) and 3D7 was detected for 7 participants (1.75%). All 10 haplotypes and 3D7 showed prevalence of ≥1% (Table 9.7).

Across all surveys in KE Kombewa, 5 haplotypes had a prevalence of >5% during a survey: CIG32 (>5% prevalence during Surveys 3 to 7, up to 11.31% at Survey 4), CIG33 (>5% prevalence during Surveys 3 to 7, up to 10.28% at Survey 6), CIG35 (>5% prevalence during Surveys 4 to 6, up to 7.29% at Survey 4), CIG38 (>5% prevalence during Surveys 3 to 6, up to 7.79% at Survey 4), and CIG39 (5.03% prevalence during Survey 4). CIG32 was the most prevalent haplotype across most surveys (Surveys 3, 4, 6, and 9).

The prevalence of 3D7 haplotype fluctuated across surveys, where the highest prevalence was observed during Survey 9 (1.75%) and was not detected during Survey 7 (0%). Other haplotypes detected in KE Kombewa did not show any clear trend in prevalence across surveys, except for the following (Table 14.8.2.3):

- CIG32 showed a decrease in prevalence from Survey 6 (10.78%) through Survey 9 (3.74%)
- CIG33 showed a quick decrease in prevalence from Survey 6 (10.28%) through Survey 9 (3.74%)
- CIG35 showed a decrease in prevalence from Survey 6 (7.02%) through Survey 9 (2.99%)
- CIG38 showed a decrease in prevalence from Survey 6 (6.02%) through Survey 9 (2.00%).

3D7 haplotype was more prevalent in GH Kintampo than KE Kombewa.

Table 9.6 Haplotype prevalence by site - Analysis Set, Survey 8

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	0	398	0.00	0	0.92
	CIG11	1	398	0.25	0.01	1.39
	CIG31	1	398	0.25	0.01	1.39
	CIG32	12	398	3.02	1.57	5.21
	CIG33	4	398	1.01	0.27	2.55
	CIG34	3	398	0.75	0.16	2.19
	CIG12	2	398	0.50	0.06	1.8
	CIG35	5	398	1.26	0.41	2.91
	CIG36	3	398	0.75	0.16	2.19
	CIG37	3	398	0.75	0.16	2.19
	CIG13	2	398	0.50	0.06	1.8
KE_Kombewa	3D7	3	402	0.75	0.15	2.17
	CIG32	10	402	2.49	1.2	4.53

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
	CIG38	14	402	3.48	1.92	5.77
	CIG39	15	402	3.73	2.1	6.08
	CIG33	12	402	2.99	1.55	5.16
	CIG310	5	402	1.24	0.41	2.88
	CIG35	10	402	2.49	1.2	4.53
	CIG36	9	402	2.24	1.03	4.21
	CIG21	7	402	1.74	0.7	3.55
	CIG37	8	402	1.99	0.86	3.88
	CIG22	6	402	1.49	0.55	3.22

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.3.1 (08APR2025 23:42 GMT)

Table 9.7 Haplotype prevalence by site - Analysis Set, Survey 9

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
GH_Kintampo	3D7	1	400	0.25	0.01	1.38
	CIG315	2	400	0.50	0.06	1.79
	CIG311	2	400	0.50	0.06	1.79
	CIG32	3	400	0.75	0.15	2.18
	CIG38	5	400	1.25	0.41	2.89
	CIG312	3	400	0.75	0.15	2.18
	CIG39	3	400	0.75	0.15	2.18
	CIG33	4	400	1.00	0.27	2.54
	CIG14	2	400	0.50	0.06	1.79
	CIG310	5	400	1.25	0.41	2.89
	CIG34	3	400	0.75	0.15	2.18
KE_Kombewa	3D7	7	401	1.75	0.7	3.56
	CIG315	4	401	1.00	0.27	2.53
	CIG32	15	401	3.74	2.11	6.09
	CIG38	8	401	2.00	0.87	3.89
	CIG312	4	401	1.00	0.27	2.53
	CIG39	13	401	3.24	1.74	5.48
	CIG33	15	401	3.74	2.11	6.09
	CIG310	4	401	1.00	0.27	2.53
	CIG35	12	401	2.99	1.56	5.17
	CIG24	5	401	1.25	0.41	2.89
	CIG22	6	401	1.50	0.55	3.23

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.3.1 (08APR2025 23:42 GMT)

9.3.2. Sensitivity Analysis for Prevalence of *Plasmodium falciparum* (by site)

9.3.2.1. Sensitivity using worst case scenario

The sensitivity analysis using worst-case scenario for the prevalence of detected *P. falciparum* haplotypes by site during Surveys 3, 4, 5, 6, and 7 is presented in the IR dated 26 November 2024.

The sensitivity analysis using worst-case scenario for the prevalence of detected *P. falciparum* haplotypes by site during Surveys 8 and 9 is provided in Table 14.6.4.1 (presented in Table 9.8) and Table 14.7.4.1 (presented in Table 9.9), respectively.

GH Kintampo:

Survey 3: Among all detected haplotypes, CIG39 was the most prevalent haplotype, with a prevalence of 15.46% as compared to 14.46% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.1.4.1).

Survey 4: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 15.83% as compared to 13.32% for 3D7 haplotype. There were 8 other detected haplotypes, each with a prevalence of >10% (see Table 14.2.4.1).

Survey 5: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 12.28% as compared to 12.03% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.3.4.1).

Survey 6: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 8.46% as compared to 5.22% for 3D7 haplotype. There were 3 other detected haplotypes, each with a prevalence of >5% (see Table 14.4.4.1).

Survey 7: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 7.02% as compared to 6.77% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >5% (see Table 14.5.4.1).

Survey 8: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 7.04% as compared to 4.02% for 3D7 haplotype. There were 2 other detected haplotypes, each with a prevalence of >5% (Table 9.8).

Survey 9: Among all detected haplotypes, CIG38 and CIG310 were the most prevalent haplotypes with a prevalence of 5.75%, each as compared to 4.75% for 3D7 haplotype. There were 8 other detected haplotypes, each with a prevalence of ≥5% (Table 9.9).

Under the “worst case scenario” assumption for sensitivity analysis at each time point, CIG32 was the most prevalent haplotype across most surveys (Surveys 4 to 8). 3D7 haplotype prevalence was highest in Survey 3 (14.46%) and lowest in Survey 8 (4.02%) for GH Kintampo.

KE Kombewa:

Survey 3: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 18.25% as compared to 10.00% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.1.4.1).

Survey 4: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 19.85% as compared to 8.79% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.2.4.1).

Survey 5: Among all detected haplotypes, CIG33 was the most prevalent haplotype with a prevalence of 19.65% as compared to 12.19% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.3.4.1).

Survey 6: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 19.30% as compared to 9.02% for 3D7 haplotype. There were 9 other detected haplotypes, each with a prevalence of >10% (see Table 14.4.4.1).

Survey 7: Among all detected haplotypes, CIG33 was the most prevalent haplotype with a prevalence of 12.75% as compared to 6.75% of 3D7 haplotype. There were 4 other detected haplotypes with prevalence of >10% (see Table 14.5.4.1).

Survey 8: Among all detected haplotypes, CIG39 was the most prevalent haplotype with a prevalence of 12.19% as compared to 9.20% of 3D7 haplotype. There were 7 other detected haplotypes with prevalence of >10% (Table 9.8).

Survey 9: Among all detected haplotypes, CIG32 and CIG33 were the most prevalent haplotypes with a prevalence of 13.72% each, as compared to 11.72% of 3D7 haplotype. There were 8 other detected haplotypes with prevalence of >10% (Table 9.9).

Under the “worst case scenario” assumption for sensitivity analysis at each time point, the most prevalent haplotypes were CIG32 (all except for Surveys 5, 7, and 8) and CIG33 (noted for Surveys 5, 7, and 9). 3D7 haplotype prevalence was highest in Survey 5 (12.19%) and lowest in Survey 7 (6.75%) for KE Kombewa.

Table 9.8 Haplotype prevalence by site - Sensitivity on worst case scenario - Analysis Set, Survey 8

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	16	398	4.02	2.32	6.45
	CIG11	17	398	4.27	2.51	6.75
	CIG31	17	398	4.27	2.51	6.75
	CIG32	28	398	7.04	4.73	10.01
	CIG33	20	398	5.03	3.1	7.65
	CIG34	19	398	4.77	2.9	7.35
	CIG12	18	398	4.52	2.7	7.05
	CIG35	21	398	5.28	3.3	7.95
	CIG36	19	398	4.77	2.9	7.35
	CIG37	19	398	4.77	2.9	7.35
	CIG13	18	398	4.52	2.7	7.05

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
KE_Kombewa	3D7	37	402	9.20	6.56	12.46
	CIG32	44	402	10.95	8.07	14.41
	CIG38	48	402	11.94	8.94	15.52
	CIG39	49	402	12.19	9.16	15.79
	CIG33	46	402	11.44	8.5	14.97
	CIG310	39	402	9.70	6.99	13.02
	CIG35	44	402	10.95	8.07	14.41
	CIG36	43	402	10.70	7.85	14.14
	CIG21	41	402	10.20	7.42	13.58
	CIG37	42	402	10.45	7.63	13.86
	CIG22	40	402	9.95	7.2	13.3

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.4.1 (08APR2025 23:42 GMT)

Table 9.9 Haplotype prevalence by site - Sensitivity on worst case scenario - Analysis Set, Survey 9

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
GH_Kintampo	3D7	19	400	4.75	2.88	7.32
	CIG315	20	400	5.00	3.08	7.62
	CIG311	20	400	5.00	3.08	7.62
	CIG32	21	400	5.25	3.28	7.91
	CIG38	23	400	5.75	3.68	8.5
	CIG312	21	400	5.25	3.28	7.91
	CIG39	21	400	5.25	3.28	7.91
	CIG33	22	400	5.50	3.48	8.21
	CIG14	20	400	5.00	3.08	7.62
	CIG310	23	400	5.75	3.68	8.5
	CIG34	21	400	5.25	3.28	7.91
KE_Kombewa	3D7	47	401	11.72	8.74	15.28
	CIG315	44	401	10.97	8.09	14.45
	CIG32	55	401	13.72	10.5	17.48
	CIG38	48	401	11.97	8.96	15.56
	CIG312	44	401	10.97	8.09	14.45
	CIG39	53	401	13.22	10.06	16.93
	CIG33	55	401	13.72	10.5	17.48
	CIG310	44	401	10.97	8.09	14.45
	CIG35	52	401	12.97	9.84	16.66
	CIG24	45	401	11.22	8.3	14.73
	CIG22	46	401	11.47	8.52	15

CI = confidence interval; GH = Ghana; KE = Kenya.
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.4.1 (08APR2025 23:42 GMT)

9.3.2.2. Sensitivity using threshold of 50 reads

The sensitivity analysis using threshold of 50 reads for the prevalence of detected *P. falciparum* haplotypes by site during Surveys 3 to 7, is presented in the IR dated 26 November 2024. The sensitivity analysis using threshold of 50 reads for the prevalence of detected *P. falciparum* haplotypes by site during Surveys 8 and 9 is provided in Table 14.6.5.2 (presented in Table 9.10) and Table 14.7.5.2 (presented in Table 9.11), respectively.

GH Kintampo:

Survey 3: Among all detected haplotypes, CIG39 was the most prevalent haplotype with a prevalence of 6.73% as compared to 5.99% for 3D7 haplotype. There were 2 other detected haplotypes, each with a prevalence of >5% (see Table 14.1.5.2).

Survey 4: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 7.79% as compared to 5.53% for 3D7 haplotype. There were no other detected haplotypes with a prevalence of >5% (see Table 14.2.5.2).

Survey 5: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 2.76% as compared to 2.01% for 3D7 haplotype. There were no detected haplotypes with a prevalence of >5% (see Table 14.3.5.2).

Survey 6: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 5.47% as compared to 2.49% for 3D7 haplotype. There were no other detected haplotypes with a prevalence of >5% (see Table 14.4.5.2).

Survey 7: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 2.26% as compared to 1.75% for 3D7 haplotype. There were no detected haplotypes with a prevalence of >5% (see Table 14.5.5.2).

Survey 8: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 3.02%, while 3D7 haplotype was not detected. There were no detected haplotypes with a prevalence of >5%, while a total of 3 haplotypes showed prevalence of >1% (Table 9.10).

Survey 9: Among all detected haplotypes, CIG38 and CIG310 were the most prevalent haplotypes with a prevalence of 1.25%, each as compared to 0.25% for 3D7 haplotype. There were no detected haplotypes with a prevalence of >5%, while a total of 3 haplotypes showed prevalence of ≥1% (Table 9.11).

The results of sensitivity analysis using threshold of 50 reads in Ghana showed highest prevalence for CIG32 haplotype across all surveys except Survey 3 and Survey 9. The prevalence of 3D7 haplotype was highest in Survey 3 (5.99%) and was not detected during Survey 8.

KE Kombewa:

Survey 3: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 8.75% as compared to 1.00% for 3D7 haplotype. There was 1 other detected haplotype (CIG33 with 7.50%) with a prevalence of >5% (see Table 14.1.5.2).

Survey 4: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 9.80% as compared to 0.25% for 3D7 haplotype. There were 3 other detected haplotypes, each with a prevalence of >5% (see Table 14.2.5.2).

Survey 5: Among all detected haplotypes, CIG33 was the most prevalent haplotype with a prevalence of 7.96% as compared to 0.75% for 3D7 haplotype. There were 2 other detected haplotypes with a prevalence of >5% (see Table 14.3.5.2).

Survey 6: Among all detected haplotypes, CIG32 was the most prevalent haplotype with a prevalence of 10.53% as compared to 0.50% for 3D7 haplotype. There were 3 other detected haplotypes, each with a prevalence of >5% (see Table 14.4.5.2).

Survey 7: Among all detected haplotypes, CIG33 was the most prevalent haplotype (5.50%). 3D7 haplotype was not detected. There was 1 other detected haplotype with a prevalence of >5% (see Table 14.5.5.2).

Survey 8: Among all detected haplotypes, CIG39 was the most prevalent haplotype (3.48%) as compared to 0.75% for 3D7 haplotype. There were no haplotypes with a prevalence of >5% (Table 9.10).

Survey 9: Among all detected haplotypes, CIG32 and CIG33 were the most prevalent haplotypes (3.49%, each) as compared to 1.50% for 3D7 haplotype. There were no haplotypes with a prevalence of >5% (Table 9.11).

The results of sensitivity analysis using threshold of 50 reads in KE Kombewa showed that CIG32 haplotype had highest prevalence across Surveys 3, 4, 6, and 9, while CIG33 haplotype had highest prevalence across Surveys 5, 7, and 9. The prevalence of 3D7 haplotype was highest in Survey 9 (1.50%) and was not detected during Survey 7.

Table 9.10 Haplotype prevalence by site - Sensitivity on threshold of 50 reads - Analysis Set, Survey 8

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	0	398	0.00	0	0.92
	CIG11	1	398	0.25	0.01	1.39
	CIG31	1	398	0.25	0.01	1.39
	CIG32	12	398	3.02	1.57	5.21
	CIG33	4	398	1.01	0.27	2.55
	CIG34	3	398	0.75	0.16	2.19
	CIG12	2	398	0.50	0.06	1.8
	CIG35	5	398	1.26	0.41	2.91
	CIG36	3	398	0.75	0.16	2.19
	CIG37	3	398	0.75	0.16	2.19
	CIG13	2	398	0.50	0.06	1.8

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
KE_Kombewa	3D7	3	402	0.75	0.15	2.17
	CIG32	9	402	2.24	1.03	4.21
	CIG38	12	402	2.99	1.55	5.16
	CIG39	14	402	3.48	1.92	5.77
	CIG33	12	402	2.99	1.55	5.16
	CIG310	5	402	1.24	0.41	2.88
	CIG35	9	402	2.24	1.03	4.21
	CIG36	8	402	1.99	0.86	3.88
	CIG21	7	402	1.74	0.7	3.55
	CIG37	8	402	1.99	0.86	3.88
	CIG22	6	402	1.49	0.55	3.22

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 50 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.5.2 (08APR2025 23:42 GMT)

Table 9.11 Haplotype prevalence by site - Sensitivity on threshold of 50 reads - Analysis Set, Survey 9

					95% CI	
Site	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
GH_Kintampo	3D7	1	400	0.25	0.01	1.38
	CIG315	2	400	0.50	0.06	1.79
	CIG311	2	400	0.50	0.06	1.79
	CIG32	3	400	0.75	0.15	2.18
	CIG38	5	400	1.25	0.41	2.89
	CIG312	3	400	0.75	0.15	2.18
	CIG39	3	400	0.75	0.15	2.18
	CIG33	4	400	1.00	0.27	2.54
	CIG14	2	400	0.50	0.06	1.79
	CIG310	5	400	1.25	0.41	2.89
	CIG34	3	400	0.75	0.15	2.18
KE_Kombewa	3D7	6	401	1.50	0.55	3.23
	CIG315	4	401	1.00	0.27	2.53
	CIG32	14	401	3.49	1.92	5.79
	CIG38	7	401	1.75	0.7	3.56
	CIG312	4	401	1.00	0.27	2.53
	CIG39	12	401	2.99	1.56	5.17
	CIG33	14	401	3.49	1.92	5.79
	CIG310	4	401	1.00	0.27	2.53
	CIG35	11	401	2.74	1.38	4.86
	CIG24	5	401	1.25	0.41	2.89
	CIG22	6	401	1.50	0.55	3.23

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 50 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.5.2 (08APR2025 23:42 GMT)

9.3.3. Longitudinal Estimates of *P. falciparum* Haplotype Frequency by site

The frequency of *P. falciparum* haplotypes by site, i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Survey 3 (Table 14.1.2.1), Survey 4 (Table 14.2.2.1), Survey 5 (Table 14.3.2.1), Survey 6 (Table 14.4.2.1), and Survey 7 (Table 14.5.2.1) is presented in the IR dated 26 November 2024. The frequency of *P. falciparum* haplotypes by site; i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Survey 8 and Survey 9 are provided in Table 14.6.2.1 (presented in Table 9.12) and Table 14.7.2.1 (presented in Table 9.13), respectively.

A graphical representation of the frequency of *P. falciparum* haplotypes by site is provided in Figure 14.6.2.2 and Figure 14.7.2.2 for Survey 8 and Survey 9, respectively.

GH Kintampo:

Survey 3: Among all detected haplotypes, the most frequent haplotype was CIG39 with a frequency of 8.82% as compared to 7.35% for 3D7 haplotype. CIG32, CIG33, and CIG38 were other detected haplotypes, each with a frequency of >5% (Table 14.1.2.1).

Survey 4: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 11.22% as compared to 7.82% for 3D7 haplotype. CIG33, CIG38, and CIG39 were other detected haplotypes, each with a frequency of >5% (Table 14.2.2.1).

Survey 5: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 9.09% as compared to 8.26% for 3D7 haplotype. CIG21, CIG38, and CIG39 were other detected haplotypes, each with a frequency of >5% (Table 14.3.2.1).

Survey 6: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 10.50% as compared to 4.57% for 3D7 haplotype. CIG122 and CIG38 were other detected haplotypes, each with a frequency of >5% (Table 14.4.2.1).

Survey 7: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 10.59% as compared to 9.41% for 3D7 haplotype. CIG21, CIG33, CIG38, CIG39, and CIG122 were other detected haplotypes, each with a frequency of >5% (Table 14.5.2.1).

Survey 8: Among all detected haplotypes, the most frequent haplotype was CIG32 (18.75% frequency), while haplotype 3D7 was not detected. CIG33 and CIG35 were other detected haplotypes, each with a frequency of >5% (Table 9.12).

Survey 9: Among all detected haplotypes, the most frequent haplotypes were CIG38 and CIG310 (7.04% frequency, each) as compared to 1.41% for 3D7 haplotype. CIG33 was the only other haplotype with frequency of >5% (Table 9.13).

Across all surveys in GH Kintampo, 8 haplotypes had a frequency of >5% during any particular survey: CIG21, CIG32, CIG33, CIG35, CIG38, CIG39, CIG122, and CIG310. CIG32 was the most frequently detected haplotype across most surveys (Surveys 4, 5, 6, 7, and 8).

The frequency of 3D7 haplotype fluctuated across surveys, where the highest frequency was observed during Survey 7 (9.41%) and was not detected during Survey 8. Other haplotypes detected in GH Kintampo did not show any clear trend in frequency across surveys.

KE Kombewa:

Survey 3: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 10.25% as compared to 1.11% for 3D7 haplotype. CIG33, CIG35, and CIG38 were the other detected haplotypes, each with a frequency of >5% (Table 14.1.2.1).

Survey 4: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 12.36% as compared to 0.27% for 3D7 haplotype. CIG33, CIG35, CIG38, CIG39, and CIG310 were other detected haplotypes, each with a frequency of >5% (Table 14.2.2.1).

Survey 5: Among all detected haplotypes, the most frequent haplotype was CIG33 with a frequency of 11.78% as compared to 1.35% for 3D7 haplotype. CIG32, CIG35, CIG38, and CIG39 were other detected haplotypes, each with a frequency of >5% (Table 14.3.2.1).

Survey 6: Among all detected haplotypes, the most frequent haplotype was CIG32 with a frequency of 12.01% as compared to 0.56% for 3D7 haplotype. CIG33, CIG35, and CIG38 were other detected haplotypes, each with a frequency of >5% (Table 14.4.2.1).

Survey 7: Among all detected haplotypes, the most frequent haplotype was CIG33 (10.20%). 3D7 haplotype was not detected. CIG32, CIG35, CIG38, and CIG39 were other detected haplotypes, each with a frequency of >5% (Table 14.5.2.1).

Survey 8: Among all detected haplotypes, the most frequent haplotype was CIG39 with a frequency of 7.28% as compared to 1.46% for 3D7 haplotype. CIG33 and CIG38 were other haplotypes with a frequency of >5% (Table 9.12).

Survey 9: Among all detected haplotypes, the most frequent haplotypes were CIG32 and CIG33 with a frequency of 8.93%, each as compared to 4.17% for 3D7 haplotype. CIG35 and CIG39 were other haplotypes with a frequency of >5% (Table 9.13).

Across all surveys in KE Kombewa, 6 haplotypes had a frequency of >5% during a survey: CIG32, CIG33, CIG35, CIG38, CIG39, CIG310. CIG32 haplotype was most

frequently detected during Surveys 3, 4, 6, and 9, while CIG33 haplotype was most frequently detected during Surveys 5, 7, and 9.

The frequency of 3D7 haplotype fluctuated across surveys, where the highest frequency was observed during Survey 9 (4.17%) and was not detected during Survey 7. Other haplotypes detected in KE Kombewa did not show any clear trend across surveys, except for CIG39 haplotype showing a gradual increase in frequency from Survey 3 (3.88%) to Survey 9 (7.74%).

Table 9.12 Haplotype frequency by site - Analysis Set, Survey 8

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	0	64	0.00	0	5.6
	CIG32	12	64	18.75	10.08	30.46
	CIG35	5	64	7.81	2.59	17.3
	CIG33	4	64	6.25	1.73	15.24
	CIG34	3	64	4.69	0.98	13.09
	CIG36	3	64	4.69	0.98	13.09
	CIG37	3	64	4.69	0.98	13.09
	CIG12	2	64	3.13	0.38	10.84
	CIG13	2	64	3.13	0.38	10.84
	CIG11	1	64	1.56	0.04	8.4
	CIG31	1	64	1.56	0.04	8.4
KE_Kombewa	3D7	3	206	1.46	0.3	4.2
	CIG39	15	206	7.28	4.13	11.73
	CIG38	14	206	6.80	3.77	11.14
	CIG33	13	206	6.31	3.4	10.55
	CIG32	10	206	4.85	2.35	8.75
	CIG35	10	206	4.85	2.35	8.75
	CIG36	9	206	4.37	2.02	8.13
	CIG21	8	206	3.88	1.69	7.51
	CIG37	8	206	3.88	1.69	7.51
	CIG22	6	206	2.91	1.08	6.23
	CIG310	5	206	2.43	0.79	5.57

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.2.1 (08APR2025 23:38 GMT)

Table 9.13 Haplotype frequency by site - Analysis Set, Survey 9

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	1	71	1.41	0.04	7.6
	CIG38	5	71	7.04	2.33	15.67
	CIG310	5	71	7.04	2.33	15.67
	CIG33	4	71	5.63	1.56	13.8
	CIG32	3	71	4.23	0.88	11.86

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
	CIG312	3	71	4.23	0.88	11.86
	CIG39	3	71	4.23	0.88	11.86
	CIG34	3	71	4.23	0.88	11.86
	CIG315	2	71	2.82	0.34	9.81
	CIG311	2	71	2.82	0.34	9.81
	CIG14	2	71	2.82	0.34	9.81
KE_Kombewa	3D7	7	168	4.17	1.69	8.4
	CIG32	15	168	8.93	5.08	14.3
	CIG33	15	168	8.93	5.08	14.3
	CIG39	13	168	7.74	4.18	12.87
	CIG35	12	168	7.14	3.75	12.14
	CIG38	8	168	4.76	2.08	9.17
	CIG24	6	168	3.57	1.32	7.61
	CIG22	6	168	3.57	1.32	7.61
	CIG315	4	168	2.38	0.65	5.98
	CIG312	4	168	2.38	0.65	5.98
	CIG310	4	168	2.38	0.65	5.98

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.2.1 (08APR2025 23:38 GMT)

9.3.4. Sensitivity analysis for Frequency of *Plasmodium falciparum* haplotypes using threshold of 50 reads (by site)

The sensitivity analysis using threshold of 50 reads for the frequency of detected *P. falciparum* haplotypes by site during Survey 3 (Table 14.1.5.1), Survey 4 (Table 14.2.5.1), Survey 5 (Table 14.3.5.1), Survey 6 (Table 14.4.5.1), and Survey 7 (Table 14.5.5.1) is presented in the IR dated 26 November 2024. The sensitivity analysis using threshold of 50 reads for the frequency of detected *P. falciparum* haplotypes by site during Surveys 8 and 9 is provided in Table 14.6.5.1 (presented in Table 9.14) and Table 14.7.5.1 (presented in Table 9.15), respectively.

GH Kintampo:

Survey 3: Among all detected haplotypes, CIG39 was the most frequently detected haplotype with a frequency of 8.63% as compared to 7.67% for 3D7 haplotype. There were 3 other detected haplotypes, each with a frequency of >5% (see Table 14.1.5.1).

Survey 4: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 11.40% as compared to 8.09% for 3D7 haplotype. There were 3 other detected haplotypes, each with a frequency of >5% (see Table 14.2.5.1).

Survey 5: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 10.68% as compared to 7.77% for 3D7 haplotype. There were 3 other detected haplotypes, each with a frequency of >5% (see Table 14.3.5.1).

Survey 6: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 10.09% as compared to 4.59% for 3D7 haplotype. There were 2 other detected haplotypes, each with a frequency of >5% (see Table 14.4.5.1).

Survey 7: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 11.11% as compared to 8.64% for 3D7 haplotype. There were 4 other detected haplotypes, each with a frequency of >5% (see Table 14.5.5.1).

Survey 8: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 19.05%, while 3D7 haplotype was not detected. There were 2 other haplotypes detected with a frequency of >5% (Table 9.14).

Survey 9: Among all detected haplotypes, CIG38 and CIG310 were the most frequently detected haplotype with a frequency of 7.04%, each as compared to 1.41% for 3D7 haplotype. There was 1 other haplotype detected with a frequency of >5% (Table 9.15).

The results of sensitivity analysis using threshold of 50 reads in GH Kintampo showed highest frequency for CIG32 across all surveys except Survey 3 and Survey 9. 3D7 haplotype frequency was highest during Survey 7 (8.64%) and was not detected during Survey 8.

KE Kombewa:

Survey 3: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 10.39% as compared to 1.19% for 3D7 haplotype. There were 3 other detected haplotypes, each with a frequency of >5% (see Table 14.1.5.1).

Survey 4: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 11.27% as compared to 0.29% for 3D7 haplotype. There were 6 other detected haplotypes, each with a frequency of >5% (see Table 14.2.5.1).

Survey 5: Among all detected haplotypes, CIG33 was the most frequently detected haplotype with a frequency of 13.31% as compared to 1.21% for 3D7 haplotype. There were 5 other detected haplotypes, each with a frequency of >5% (see Table 14.3.5.1).

Survey 6: Among all detected haplotypes, CIG32 was the most frequently detected haplotype with a frequency of 12.14% as compared to 0.58% for 3D7 haplotype. There were 3 other detected haplotypes, each with a frequency of >5% (see Table 14.4.5.1).

Survey 7: Among all detected haplotypes, CIG33 was the most frequently detected haplotype with a frequency of 10.18%, while 3D7 haplotype was not detected. There were 4 other detected haplotypes, each with a frequency of >5% (see Table 14.5.5.1).

Survey 8: Among all detected haplotypes, CIG39 was the most frequently detected haplotype with a frequency of 7.00% as compared to 1.50% for 3D7 haplotype. There were 2 other haplotypes, each detected with a frequency of >5% (Table 9.14).

Survey 9: Among all detected haplotypes, CIG32 and CIG33 were the most frequently detected haplotype with a frequency of 8.81%, each as compared to 3.77% for 3D7 haplotype. There were 2 other haplotypes, each detected with a frequency of >5% (Table 9.15).

The results of sensitivity analysis using threshold of 50 reads at KE Kombewa showed highest frequency for CIG32 (Surveys 3, 4, 6, and 9) and CIG33 (Surveys 5, 7, and 9) across all surveys in the sensitivity analysis. 3D7 haplotype frequency was highest in Survey 9 (3.77%) and was not detected during Survey 7.

Table 9.14 Haplotype frequency by site - Sensitivity on threshold of 50 reads - Analysis Set, Survey 8

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	0	63	0.00	0	5.69
	CIG32	12	63	19.05	10.25	30.91
	CIG35	5	63	7.94	2.63	17.56
	CIG33	4	63	6.35	1.76	15.47
	CIG34	3	63	4.76	0.99	13.29
	CIG36	3	63	4.76	0.99	13.29
	CIG37	3	63	4.76	0.99	13.29
	CIG12	2	63	3.17	0.39	11
	CIG13	2	63	3.17	0.39	11
	CIG11	1	63	1.59	0.04	8.53
	CIG31	1	63	1.59	0.04	8.53
KE_Kombewa	3D7	3	200	1.50	0.31	4.32
	CIG39	14	200	7.00	3.88	11.47
	CIG33	13	200	6.50	3.51	10.86
	CIG38	12	200	6.00	3.14	10.25
	CIG32	9	200	4.50	2.08	8.37
	CIG35	9	200	4.50	2.08	8.37
	CIG36	8	200	4.00	1.74	7.73
	CIG21	8	200	4.00	1.74	7.73
	CIG37	8	200	4.00	1.74	7.73
	CIG22	6	200	3.00	1.11	6.42
	CIG310	5	200	2.50	0.82	5.74

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 50 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.5.1 (08APR2025 23:38 GMT)

Table 9.15 Haplotype frequency by site - Sensitivity on threshold of 50 reads - Analysis Set, Survey 9

					95% CI	
Site	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	3D7	1	71	1.41	0.04	7.6
	CIG38	5	71	7.04	2.33	15.67
	CIG310	5	71	7.04	2.33	15.67
	CIG33	4	71	5.63	1.56	13.8
	CIG32	3	71	4.23	0.88	11.86
	CIG312	3	71	4.23	0.88	11.86
	CIG39	3	71	4.23	0.88	11.86
	CIG34	3	71	4.23	0.88	11.86
	CIG315	2	71	2.82	0.34	9.81
	CIG311	2	71	2.82	0.34	9.81
	CIG14	2	71	2.82	0.34	9.81
KE_Kombewa	3D7	6	159	3.77	1.4	8.03
	CIG32	14	159	8.81	4.9	14.33
	CIG33	14	159	8.81	4.9	14.33
	CIG39	12	159	7.55	3.96	12.81
	CIG35	11	159	6.92	3.5	12.04
	CIG38	7	159	4.40	1.79	8.86
	CIG24	6	159	3.77	1.4	8.03
	CIG22	6	159	3.77	1.4	8.03
	CIG315	4	159	2.52	0.69	6.32
	CIG312	4	159	2.52	0.69	6.32
	CIG310	4	159	2.52	0.69	6.32

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 50 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.5.1 (08APR2025 23:38 GMT)

9.4. Results of secondary analyses

9.4.1. Longitudinal estimates of *P. falciparum* haplotype prevalence by age group, gender and RTS,S/AS01$_E$ vaccination status

For participants who were infected with specific *P. falciparum* haplotypes, the prevalence of *P. falciparum* haplotypes by site, age group, gender and by vaccination status is described below.

9.4.1.1. Site-wise prevalence estimation of *P. falciparum* haplotypes by gender

For participants who were infected with the specific *P. falciparum* haplotypes, the prevalence of those detected *P. falciparum* haplotypes by site and gender, i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Survey 3 (Table 14.1.3.4), Survey 4 (Table 14.2.3.4), Survey 5 (Table 14.3.3.4), Survey 6 (Table 14.4.3.4), and Survey 7 (Table 14.5.3.4) is presented in the IR dated

26 November 2024. The prevalence of *P. falciparum* haplotypes by site and according to gender, i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Surveys 8 and 9 is provided in Table 14.6.3.4 (presented in Table 9.16) and in Table 14.7.3.4 (presented in Table 9.17), respectively.

A graphical representation of prevalence of *P. falciparum* haplotypes according to gender during Survey 8 is provided in Figure 14.6.3.5 (GH Kintampo) and Figure 14.6.3.6 (KE Kombewa), while prevalence according to gender during Survey 9 is provided in Figure 14.7.3.5 (GH Kintampo) and Figure 14.7.3.6 (KE Kombewa).

GH Kintampo:

Female participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG38 and CIG39 were present in >5% of the participants, individually. The most prevalent haplotypes were 3D7 and CIG38 (6.95%, each) (see Table 14.1.3.4).

Survey 4: Among all detected haplotypes, the most prevalent haplotypes were CIG32 and CIG38, each with a prevalence of 6.08% as compared to 4.97% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (see Table 14.2.3.4).

Survey 5: No detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most prevalent haplotype was CIG32 with a prevalence of 3.52% as compared to 2.51% for 3D7 haplotype (see Table 14.3.3.4).

Survey 6: Among all detected haplotypes, the most prevalent haplotype was CIG32 with a prevalence of 5.13% as compared to 2.56% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (see Table 14.4.3.4).

Survey 7: No detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most prevalent haplotypes were CIG21 and CIG32, each with a prevalence of 2.08% as compared to 1.56% for 3D7 haplotype (see Table 14.5.3.4).

Survey 8: No detected haplotypes were present in >5% of the participants. The most prevalent haplotype was CIG32 (3.29%), where 3 other haplotypes showed prevalence of >1%. Additionally, haplotype 3D7 was not detected among female participants during Survey 8 (Table 9.16).

Survey 9: No detected haplotypes were present in >5% of the participants. The most prevalent haplotypes were CIG32 and CIG310 (1.46%, each) as compared to 0.49% for 3D7 haplotype (detected in 1 female participant). No other haplotype showed prevalence of >1% (Table 9.17).

Among female participants in GH Kintampo, CIG32 was the most prevalent haplotype across all surveys except for Survey 3. Among female participants, 3D7 haplotype prevalence was highest during Survey 3 (6.95%; CI: 3.75 – 11.59) and was not detected during Survey 8.

Male participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG39 which had a prevalence of 7.94% as compared to 5.61% for 3D7 haplotype (see Table 14.1.3.4).

Survey 4: Among all detected haplotypes, 3D7, CIG32, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 which had a prevalence of 10.14% as compared to 6.45% of 3D7 haplotype (see Table 14.2.3.4).

Survey 5: No detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most prevalent haplotype was 3D7 (2.50%) (see Table 14.3.3.4).

Survey 6: Among all detected haplotypes, the most prevalent haplotype was CIG32 which had a prevalence of 6.28% as compared to 2.42% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (see Table 14.4.3.4).

Survey 7: No detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most prevalent haplotypes were 3D7, CIG32, and CIG33 (2.42% each) (see Table 14.5.3.4).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (2.70%), where 1 other haplotype showed prevalence of >1%. Additionally, haplotype 3D7 was not detected among male participants (Table 9.16).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG38 (2.06%), while 5 other haplotypes showed frequency of >1%. Additionally, haplotype 3D7 was not detected among male participants during Survey 9 (Table 9.17).

Among male participants in GH Kintampo, CIG32 was the most prevalent haplotype across Surveys 4, 6, 7, and 8. Among male participants, prevalence of 3D7 haplotype was highest in Survey 4 (6.45%; CI: 3.57 - 10.59) and was not detected during Surveys 8 and 9.

KE Kombewa

Female participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG310 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 7.98% as compared to 1.88% for 3D7 haplotype (see Table 14.1.3.4).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 which had prevalence of 10.05% as compared to 0.48% for 3D7 haplotype (see Table 14.2.3.4).

Survey 5: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 with a prevalence of 9.68% as compared to 1.08% for 3D7 haplotype (see Table 14.3.3.4).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 13.40% as compared to 0.48% for 3D7 haplotype (see Table 14.4.3.4).

Survey 7: No detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most prevalent haplotypes were CIG33 and CIG32 with a prevalence of 4.85%, each. 3D7 haplotype was not detected (see Table 14.5.3.4).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG38 (4.93%) as compared to 0.45% for 3D7 haplotype. All other haplotypes showed prevalence of >1% (Table 9.16).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG33 (4.52%) as compared to 1.51% for 3D7 haplotype. All other haplotypes showed prevalence of >1% (Table 9.17).

Among female participants in KE Kombewa, CIG32 was the most prevalent haplotype during Surveys 3, 4, 6, and 7, while CIG33 was the most prevalent during Surveys 5, 7, and 9. Among female participants, 3D7 haplotype prevalence was highest during Survey 3 (1.88%; CI: 0.51 - 4.74) and not detected during Survey 7 (Table 9.16).

Male participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 (10.70%). 3D7 haplotype was not detected (see Table 14.1.3.4).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 (12.70%). 3D7 haplotype was not detected (see Table 14.2.3.4).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 9.72% as compared to 0.93% for 3D7 haplotype (see Table 14.3.3.4).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 with a prevalence of 10.00% as compared to 0.53% for 3D7 haplotype (see Table 14.4.3.4).

Survey 7: Among all detected haplotypes, CIG32 and CIG33 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (7.22%). 3D7 haplotype was not detected (see Table 14.5.3.4).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG39 (4.47%) as compared to 1.12% for 3D7 haplotype. All other haplotypes showed prevalence of >1% (Table 9.16).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (4.46%) as compared to 1.98% for 3D7 haplotype. Additionally, 7 other haplotypes showed prevalence of >1% (Table 9.17).

Among male participants in KE Kombewa, CIG32 was the most prevalent haplotype across Surveys 3, 4, 5, and 9, while CIG33 was the most prevalent haplotype across Surveys 6 and 7. Among male participants, 3D7 haplotype prevalence was highest in Survey 9 (1.98%; CI: 0.54 - 4.99) and not detected during Surveys 3, 4, and 7.

Table 9.16 Haplotype prevalence by site according to gender- Analysis Set, Survey 8

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Female	3D7	0	213	0.00	0	1.72
		CIG31	1	213	0.47	0.01	2.59
		CIG32	7	213	3.29	1.33	6.65
		CIG15	1	213	0.47	0.01	2.59
		CIG16	1	213	0.47	0.01	2.59
		CIG114	1	213	0.47	0.01	2.59
		CIG116	1	213	0.47	0.01	2.59
		CIG33	4	213	1.88	0.51	4.74
		CIG34	2	213	0.94	0.11	3.35
		CIG35	4	213	1.88	0.51	4.74
		CIG37	3	213	1.41	0.29	4.06
	Male	3D7	0	185	0.00	0	1.97
		CIG11	1	185	0.54	0.01	2.97
		CIG32	5	185	2.70	0.88	6.19
		CIG17	1	185	0.54	0.01	2.97
		CIG34	1	185	0.54	0.01	2.97
		CIG18	1	185	0.54	0.01	2.97
		CIG19	1	185	0.54	0.01	2.97
		CIG110	1	185	0.54	0.01	2.97
		CIG12	1	185	0.54	0.01	2.97
		CIG35	1	185	0.54	0.01	2.97
		CIG36	2	185	1.08	0.13	3.85
KE_Kombewa	Female	3D7	1	223	0.45	0.01	2.47
		CIG311	4	223	1.79	0.49	4.53
		CIG32	5	223	2.24	0.73	5.15
		CIG38	11	223	4.93	2.49	8.65
		CIG39	7	223	3.14	1.27	6.36
		CIG33	6	223	2.69	0.99	5.76
		CIG310	4	223	1.79	0.49	4.53
		CIG34	4	223	1.79	0.49	4.53
		CIG35	4	223	1.79	0.49	4.53
		CIG36	5	223	2.24	0.73	5.15
		CIG26	4	223	1.79	0.49	4.53
	Male	3D7	2	179	1.12	0.14	3.98
		CIG315	2	179	1.12	0.14	3.98
		CIG31	2	179	1.12	0.14	3.98

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG32	5	179	2.79	0.91	6.4
		CIG38	3	179	1.68	0.35	4.82
		CIG39	8	179	4.47	1.95	8.62
		CIG33	6	179	3.35	1.24	7.15
		CIG35	6	179	3.35	1.24	7.15
		CIG36	4	179	2.23	0.61	5.62
		CIG21	5	179	2.79	0.91	6.4
		CIG37	5	179	2.79	0.91	6.4

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.3.4 (08APR2025 23:42 GMT)

Table 9.17 Haplotype prevalence by site according to gender- Analysis Set, Survey 9

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Female	3D7	1	206	0.49	0.01	2.67
		CIG315	1	206	0.49	0.01	2.67
		CIG111	1	206	0.49	0.01	2.67
		CIG112	1	206	0.49	0.01	2.67
		CIG311	1	206	0.49	0.01	2.67
		CIG31	1	206	0.49	0.01	2.67
		CIG32	3	206	1.46	0.3	4.2
		CIG113	1	206	0.49	0.01	2.67
		CIG33	2	206	0.97	0.12	3.46
		CIG14	2	206	0.97	0.12	3.46
		CIG310	3	206	1.46	0.3	4.2
	Male	3D7	0	194	0.00	0	1.88
		CIG315	1	194	0.52	0.01	2.84
		CIG311	1	194	0.52	0.01	2.84
		CIG118	1	194	0.52	0.01	2.84
		CIG314	1	194	0.52	0.01	2.84
		CIG38	4	194	2.06	0.56	5.19
		CIG312	2	194	1.03	0.13	3.67
		CIG39	2	194	1.03	0.13	3.67
		CIG33	2	194	1.03	0.13	3.67
		CIG310	2	194	1.03	0.13	3.67
		CIG34	3	194	1.55	0.32	4.45
KE_Kombewa	Female	3D7	3	199	1.51	0.31	4.34
		CIG315	2	199	1.01	0.12	3.58
		CIG32	6	199	3.02	1.11	6.45
		CIG39	7	199	3.52	1.43	7.11
		CIG33	9	199	4.52	2.09	8.41
		CIG313	2	199	1.01	0.12	3.58
		CIG34	3	199	1.51	0.31	4.34
		CIG35	6	199	3.02	1.11	6.45

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG36	3	199	1.51	0.31	4.34
		CIG24	3	199	1.51	0.31	4.34
		CIG22	3	199	1.51	0.31	4.34
	Male	3D7	4	202	1.98	0.54	4.99
		CIG315	2	202	0.99	0.12	3.53
		CIG32	9	202	4.46	2.06	8.29
		CIG23	3	202	1.49	0.31	4.28
		CIG38	7	202	3.47	1.4	7.01
		CIG312	3	202	1.49	0.31	4.28
		CIG39	6	202	2.97	1.1	6.35
		CIG33	6	202	2.97	1.1	6.35
		CIG35	6	202	2.97	1.1	6.35
		CIG24	2	202	0.99	0.12	3.53
		CIG22	3	202	1.49	0.31	4.28

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.3.4 (08APR2025 23:42 GMT)

9.4.1.2. Site-wise prevalence estimation of *Plasmodium falciparum* haplotypes by age group

For participants who were infected with the specific *P. falciparum* haplotypes, the prevalence of those detected *P. falciparum* haplotypes by site and age group i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Survey 3 (Table 14.1.3.7), Survey 4 (Table 14.2.3.7), Survey 5 (Table 14.3.3.7), Survey 6 (Table 14.4.3.7), and Survey 7 (Table 14.5.3.7) is presented in the IR dated 26 November 2024. The prevalence of *P. falciparum* haplotypes by site and according to age group i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Surveys 8 and 9 is provided in Table 14.6.3.7 (presented in Table 9.18) and in Table 14.7.3.7 (presented in Table 9.19), respectively.

A graphical representation of prevalence of *P. falciparum* haplotypes according to age group during Survey 8 is provided in Figure 14.6.3.8 (GH Kintampo) and Figure 14.6.3.9 (KE Kombewa), while prevalence according to age group during Survey 9 is provided in Figure 14.7.3.8 (GH Kintampo) and Figure 14.7.3.9 (KE Kombewa).

GH Kintampo:

0.5 to 1 year old participants:

Survey 3: Among all detected haplotypes, the most prevalent haplotype was CIG39 which had a prevalence of 4.95% as compared to 3.85% for 3D7 haplotype. No detected haplotypes were present in >5% of the participants (Table 14.1.3.7).

Survey 4: Among all detected haplotypes, the most prevalent haplotype was CIG32 which had a prevalence of 6.04% as compared to 3.85% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (Table 14.2.3.7).

Survey 5: Among all detected haplotypes, the most prevalent haplotype was CIG39 which had a prevalence of 2.23% as compared to 1.12% for 3D7 haplotype. No detected haplotypes were present in >5% of the participants (Table 14.3.3.7).

Survey 6: Among all detected haplotypes, the most prevalent haplotype was CIG32 which had a prevalence of 5.56% as compared to 2.22% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (Table 14.4.3.7).

Survey 7: Among all detected haplotypes, the most prevalent haplotypes were CIG32 and CIG312 (1.67% each). 3D7 haplotype was not detected. No detected haplotypes were present in >5% of the participants (Table 14.5.3.7).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (2.81%), where 1 other haplotype showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.18).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG32, CIG33, CIG34, CIG39, CIG119, and CIG310 (1.14%, each), while no other haplotype showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.19).

Among 0.5 to 1 year old participants in GH Kintampo, CIG32 was the most prevalent haplotype across all surveys except for Surveys 3 and 5, while CIG39 was the most prevalent haplotype across Surveys 3, 5, and 9. The prevalence of 3D7 haplotype was highest during Surveys 3 and 4 (3.85%; CI: 1.56 - 7.76, each) and not detected during Surveys 7, 8, and 9.

2 to 4 years old participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG39 with a prevalence of 9.13% as compared to 8.22% for 3D7 haplotype (Table 14.1.3.7).

Survey 4: Among all detected haplotypes, 3D7, CIG32, CIG33, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 10.19% as compared to 7.41% for 3D7 haplotype (Table 14.2.3.7).

Survey 5: Among all detected haplotypes, the most prevalent haplotype was CIG32 with a prevalence of 4.09% as compared to 3.64% for 3D7 haplotype. No detected haplotypes were present in >5% of the participants (Table 14.3.3.7).

Survey 6: Among all detected haplotypes, the most prevalent haplotype was CIG32, with a prevalence of 5.86% as compared to 2.70% of 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (Table 14.4.3.7).

Survey 7: Among all detected haplotypes, the most prevalent haplotype was 3D7 (3.65%). No detected haplotypes were present in >5% of the participants (Table 14.5.3.7).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (3.18%), where 2 other haplotypes showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.18).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG38 (1.79%) as compared to 0.45% for 3D7 haplotype (detected in 1 participant from the 2 to 4 years old group). Two other haplotypes showed frequency of >1% (Table 9.19).

Among 2 to 4 years old participants in GH Kintampo, CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8. The prevalence of 3D7 haplotype was highest during Survey 3 (8.22%; CI: 4.94 - 12.68) and not detected during Survey 8.

KE Kombewa

0.5 to 1 year old participants:

Survey 3: Among all detected haplotypes, CIG32 and CIG33 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 8.84% as compared to 0.55% for 3D7 haplotype (Table 14.1.3.7).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38haplotypes were present in >5% of the participants, individually. The most prevalent haplotypes were CIG32 with a prevalence of 8.89% as compared to 0.56% for 3D7 haplotype (Table 14.2.3.7).

Survey 5: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotypes were CIG32 and CIG38 and each had a prevalence of 6.56% as compared to 0.55% for 3D7 haplotype (Table 14.3.3.7).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (10.00%). 3D7 haplotype was not detected (Table 14.4.3.7).

Survey 7: Among all detected haplotypes, the most prevalent haplotype was CIG32 (4.44%). No detected haplotypes were present in >5% of the participants. 3D7 haplotype was not detected (Table 14.5.3.7).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG35 and CIG39 (3.28%, each) as compared to 0.55% for 3D7 haplotype (detected in 1 child 0.5 to 1 year of age). All other haplotypes showed prevalence of >1% (Table 9.18).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (3.87%) as compared to 2.76% for 3D7 haplotype. All other haplotypes showed prevalence of >1% (Table 9.19).

Among 0.5 to 1 year old participants in KE Kombewa, CIG32 was the most prevalent during Surveys 3, 4, 5, 7, and 9. The prevalence of 3D7 haplotype was highest in Survey 9 (2.76%; CI: 0.9 - 6.33) and was not detected in Surveys 6 and 7.

2 to 4 years old participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 9.59% as compared to 1.37% for 3D7 haplotype (Table 14.1.3.7).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG38, CIG39, and CIG310 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 (13.30%). 3D7 haplotype was not detected (Table 14.2.3.7).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 with a prevalence of 10.50% as compared to 1.37% for 3D7 haplotype (Table 14.3.3.7).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 13.70% as compared to 0.91% for 3D7 haplotype (Table 14.4.3.7).

Survey 7: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (7.73%). 3D7 haplotype was not detected (Table 14.5.3.7).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG38 and CIG39 (4.11%, each) as compared to 0.91% for 3D7 haplotype (detected in 2 children 2 to 4 years of age). All other haplotypes showed prevalence of >1% (Table 9.18).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG33 (4.55%) as compared to 0.91% for 3D7 haplotype (detected in 2 children 2 to 4 years of age). All other haplotypes showed prevalence of >1% (Table 9.19).

Among 2 to 4 years old participants in KE Kombewa, CIG32 was the most prevalent haplotype across Surveys 3, 4, and 6, while CIG33 was the most prevalent haplotype across Surveys 5, 7, and 9. The prevalence of 3D7 haplotype prevalence was highest in Surveys 3 and 5 (1.37%; CI: 0.28 - 3.95, each) and was not detected in Surveys 4 and 7.

Table 9.18 Haplotype prevalence by site according to age group- Analysis Set, Survey 8

						95% CI	
Site	Age Group	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	0.5-1Y	3D7	0	178	0.00	0	2.05
		CIG32	5	178	2.81	0.92	6.43
		CIG15	1	178	0.56	0.01	3.09
		CIG16	1	178	0.56	0.01	3.09
		CIG114	1	178	0.56	0.01	3.09
		CIG116	1	178	0.56	0.01	3.09
		CIG115	1	178	0.56	0.01	3.09
		CIG117	1	178	0.56	0.01	3.09
		CIG314	1	178	0.56	0.01	3.09
		CIG33	1	178	0.56	0.01	3.09
		CIG13	2	178	1.12	0.14	4
	2-4Y	3D7	0	220	0.00	0	1.66
		CIG11	1	220	0.45	0.01	2.51
		CIG31	1	220	0.45	0.01	2.51
		CIG32	7	220	3.18	1.29	6.45
		CIG17	1	220	0.45	0.01	2.51
		CIG38	1	220	0.45	0.01	2.51
		CIG33	3	220	1.36	0.28	3.93
		CIG34	2	220	0.91	0.11	3.25
		CIG35	4	220	1.82	0.5	4.59
		CIG36	2	220	0.91	0.11	3.25
		CIG37	2	220	0.91	0.11	3.25
KE_Kombewa	0.5-1Y	3D7	1	183	0.55	0.01	3.01
		CIG32	2	183	1.09	0.13	3.89
		CIG38	5	183	2.73	0.89	6.26
		CIG39	6	183	3.28	1.21	7
		CIG33	4	183	2.19	0.6	5.5
		CIG35	6	183	3.28	1.21	7
		CIG36	4	183	2.19	0.6	5.5
		CIG21	4	183	2.19	0.6	5.5
		CIG26	3	183	1.64	0.34	4.72
		CIG37	4	183	2.19	0.6	5.5
		CIG22	3	183	1.64	0.34	4.72
	2-4Y	3D7	2	219	0.91	0.11	3.26
		CIG315	3	219	1.37	0.28	3.95
		CIG311	3	219	1.37	0.28	3.95
		CIG32	8	219	3.65	1.59	7.07
		CIG38	9	219	4.11	1.9	7.66
		CIG39	9	219	4.11	1.9	7.66
		CIG33	8	219	3.65	1.59	7.07
		CIG310	3	219	1.37	0.28	3.95
		CIG35	4	219	1.83	0.5	4.61
		CIG36	5	219	2.28	0.75	5.25
		CIG37	4	219	1.83	0.5	4.61

CI = confidence interval; GH = Ghana; KE = Kenya; WHO = World Health Organisation.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date; IC = Informed consent
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.3.7 (08APR2025 23:42 GMT)

Table 9.19 Haplotype prevalence by site according to age group - Analysis Set, Survey 9

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	0.5-1Y	3D7	0	176	0.00	0	2.07
		CIG315	1	176	0.57	0.01	3.12
		CIG31	1	176	0.57	0.01	3.12
		CIG32	2	176	1.14	0.14	4.04
		CIG118	1	176	0.57	0.01	3.12
		CIG314	1	176	0.57	0.01	3.12
		CIG39	2	176	1.14	0.14	4.04
		CIG33	2	176	1.14	0.14	4.04
		CIG310	2	176	1.14	0.14	4.04
		CIG34	2	176	1.14	0.14	4.04
		CIG119	2	176	1.14	0.14	4.04
	2-4Y	3D7	1	224	0.45	0.01	2.46
		CIG315	1	224	0.45	0.01	2.46
		CIG111	1	224	0.45	0.01	2.46
		CIG112	1	224	0.45	0.01	2.46
		CIG311	2	224	0.89	0.11	3.19
		CIG32	1	224	0.45	0.01	2.46
		CIG38	4	224	1.79	0.49	4.51
		CIG312	3	224	1.34	0.28	3.86
		CIG33	2	224	0.89	0.11	3.19
		CIG310	3	224	1.34	0.28	3.86
		CIG35	2	224	0.89	0.11	3.19
KE_Kombewa	0.5-1Y	3D7	5	181	2.76	0.9	6.33
		CIG32	7	181	3.87	1.57	7.81
		CIG23	2	181	1.10	0.13	3.93
		CIG314	2	181	1.10	0.13	3.93
		CIG38	6	181	3.31	1.23	7.08
		CIG39	5	181	2.76	0.9	6.33
		CIG33	5	181	2.76	0.9	6.33
		CIG27	2	181	1.10	0.13	3.93
		CIG35	5	181	2.76	0.9	6.33
		CIG36	2	181	1.10	0.13	3.93
		CIG25	2	181	1.10	0.13	3.93
	2-4Y	3D7	2	220	0.91	0.11	3.25
		CIG315	4	220	1.82	0.5	4.59
		CIG32	8	220	3.64	1.58	7.04
		CIG312	4	220	1.82	0.5	4.59
		CIG39	8	220	3.64	1.58	7.04
		CIG33	10	220	4.55	2.2	8.2

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG310	4	220	1.82	0.5	4.59
		CIG34	3	220	1.36	0.28	3.93
		CIG35	7	220	3.18	1.29	6.45
		CIG24	4	220	1.82	0.5	4.59
		CIG22	6	220	2.73	1.01	5.84

CI = confidence interval; GH = Ghana; KE = Kenya; WHO = World Health Organisation.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date; IC = Informed consent
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.3.7 (08APR2025 23:42 GMT)

9.4.1.3. Site-wise prevalence estimation of *Plasmodium falciparum* haplotypes by RTS,S/AS01$_E$ vaccination status

For participants who were infected with the specific *P. falciparum* haplotypes, the prevalence of those detected *P. falciparum* haplotypes by site and RTS,S/AS01$_E$ vaccination status i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Survey 3 (Table 14.1.3.10), Survey 4 (Table 14.2.3.10), Survey 5 (Table 14.3.3.10), Survey 6 (Table 14.4.3.10), and Survey 7 (Table 14.5.3.10) is presented in the IR dated 26 November 2024. The prevalence of *P. falciparum* haplotypes by site and according to vaccination status i.e., the 10 most frequently detected haplotypes along with those having ≥5% prevalence during Surveys 8 and 9 is provided in Table 14.6.3.10 (presented in Table 9.20) and in Table 14.7.3.10 (presented in Table 9.21), respectively.

Haplotype prevalence by site and haplotypes according to survey are provided for vaccinated participants in Table 14.8.2.4 and for unvaccinated participants in Table 14.8.2.5.

A graphical representation of prevalence of *P. falciparum* haplotypes according to RTS,S/AS01$_E$ vaccination status during Survey 8 is provided in Figure 14.6.3.11 (GH Kintampo) and Figure 14.6.3.12 (KE Kombewa), while prevalence according to RTS,S/AS01$_E$ vaccination status during Survey 9 is provided in Figure 14.7.3.11 (GH Kintampo) and Figure 14.7.3.12 (KE Kombewa).

Prevalence across all surveys is graphically represented from the site in GH Kintampo in Figure 14.8.2.11 (Overall population), Figure 14.8.2.13 (Unvaccinated participants, Figure 9.1), and Figure 14.8.2.12 (Vaccinated participants, Figure 9.2).

Prevalence across all surveys is graphically represented from the site in KE Kombewa in Figure 14.8.2.8 (Overall population), Figure 14.8.2.10 (Unvaccinated participants, Figure 9.3), and Figure 14.8.2.9 (Vaccinated participants, Figure 9.4).

GH Kintampo:

Unvaccinated participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG39 with a prevalence of 7.23% as compared to 6.23% of 3D7 haplotype (Table 14.1.3.10).

Survey 4: Among all detected haplotypes, 3D7, CIG32 and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 8.29% as compared to 5.78% for 3D7 haplotype (Table 14.2.3.10).

Survey 5: Among all detected haplotypes, the most prevalent haplotypes were CIG32 with a prevalence of 2.76% as compared to 2.51% for 3D7 haplotype. No detected haplotypes were present in >5% of the participants (Table 14.3.3.10).

Survey 6: Among all detected haplotypes, the most prevalent haplotype was CIG32 with a prevalence of 6.18% as compared to 2.81% for 3D7 haplotype. No other detected haplotypes were present in >5% of the participants (Table 14.4.3.10).

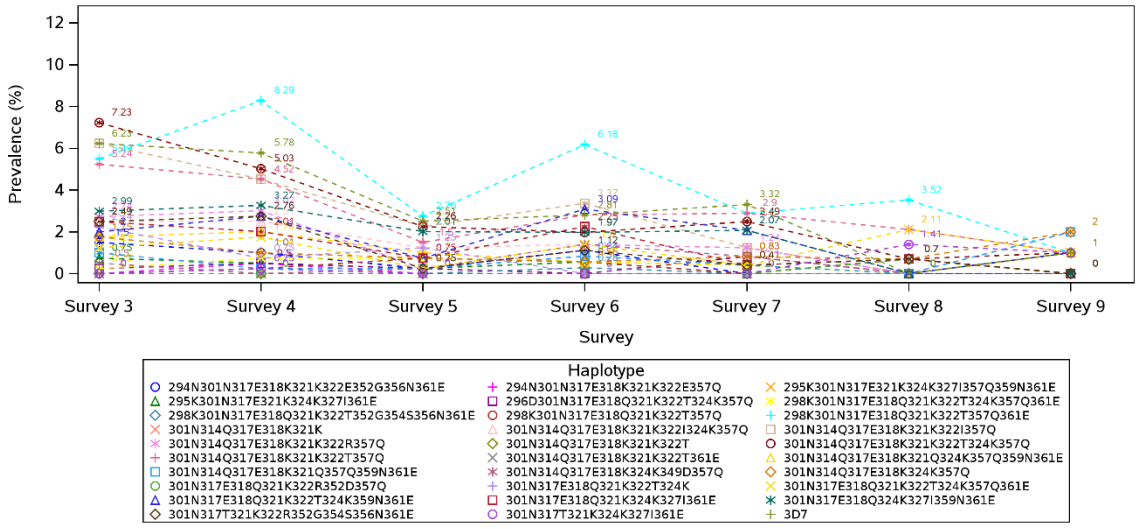
Survey 7: Among all detected haplotypes, the most prevalent haplotype was 3D7 (3.32%). No detected haplotypes were present in >5% of the participants (Table 14.5.3.10).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (3.52%), where 3 other haplotypes showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.20).

Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG34, CIG38, and CIG310 (2.00%, each) as compared to 1.00% for 3D7 haplotype (detected in 1 participant). All other haplotypes showed prevalence of 1.00% (Table 9.21).

Among unvaccinated participants in GH Kintampo, CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8. The prevalence of 3D7 haplotype was highest during Survey 3 (6.23%; CI: 4.07 - 9.07) and not detected among unvaccinated participants during Survey 8.

Figure 9.1 Haplotype prevalence in GH Kintampo over survey - Unvaccinated participants - Analysis Set



GH = Ghana.
Source: Figure 14.8.2.13 (08APR2025 23:44 GMT)

Vaccinated participants:

Survey 6: Among all detected haplotypes, the most prevalent haplotypes were CIG12, CIG32, CIG39, CIG126, and CIG127 (2.17% each), individually. No detected haplotypes were present in >5% of the participants. 3D7 haplotype was not detected (Table 14.4.3.10).

Survey 7: Among all detected haplotypes, the most prevalent haplotypes were CIG32 and CIG38 (1.27% each), individually. No detected haplotypes were present in >5% of the participants. 3D7 haplotype was not detected (Table 14.5.3.10).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (2.73%), where no other haplotype showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.20).

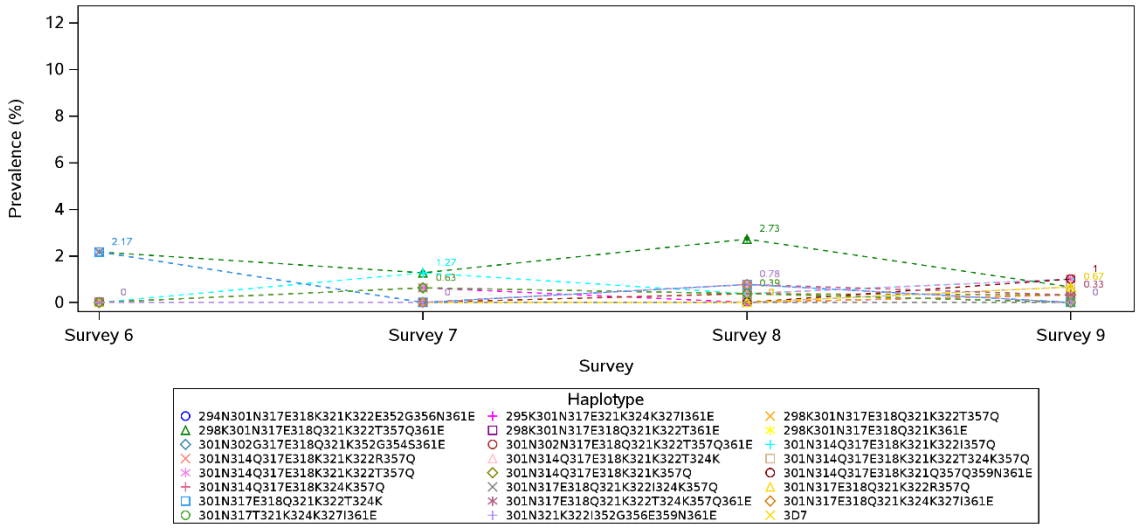
Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG33, CIG38, CIG310, and CIG312 (1.00%, each). Additionally, 3D7 haplotype was not detected (Table 9.21).

Among the participants who received RTS,S/AS01$_E$ vaccination in GH Kintampo, CIG32 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG38 was most prevalent across Surveys 7 and 9. The 3D7 haplotype was not detected among vaccinated participants during Surveys 6, 7, 8, and 9.

All other haplotypes showed low frequency across surveys, with the highest frequency observed for CIG32 during Survey 8 (2.73%).

Overall, no clear trend in prevalence of any haplotype was observed in relation to vaccination status in GH Kintampo.

Figure 9.2 Haplotype prevalence in GH Kintampo over survey - Vaccinated participants - Analysis Set



GH = Ghana.
Source: Figure 14.8.2.12 (08APR2025 23:44 GMT)

KE Kombewa

Unvaccinated participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 9.25% as compared to 1.00% for 3D7 haplotype (Table 14.1.3.10).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 11.31% as compared to 0.25% for 3D7 haplotype (Table 14.2.3.10).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 with a prevalence of 8.46% as compared to 1.00% for 3D7 haplotype (Table 14.3.3.10).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG32 with a prevalence of 11.97% as compared to 0.65% for 3D7 haplotype (Table 14.4.3.10).

Survey 7: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (6.77%). 3D7 haplotype was not detected (Table 14.5.3.10).

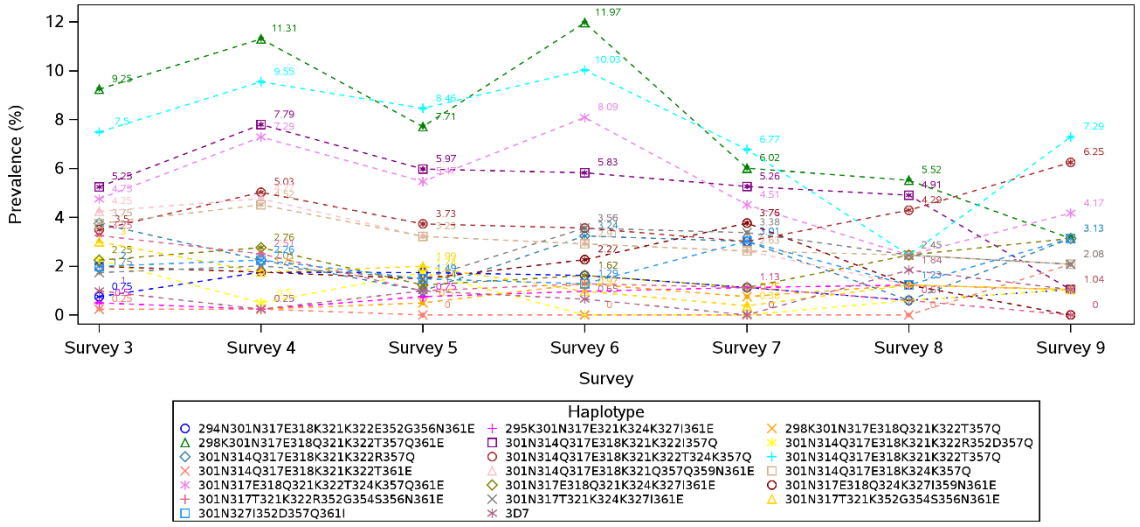
Survey 8: Among all detected haplotypes, CIG32 had a prevalence of >5% and was the most prevalent haplotype (5.52%) as compared to 1.84% for 3D7 haplotype. All other haplotypes showed prevalence of >1% (Table 9.20).

Survey 9: Among all detected haplotypes, CIG33 and CIG39 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (7.29%) as compared to 1.04% for 3D7 haplotype (detected in 1 participant), while all others had prevalence of >1% (Table 9.21).

Among unvaccinated participants in KE Kombewa, CIG32 was the most prevalent haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most prevalent during Surveys 5, 7, and 9. The prevalence of 3D7 haplotype was highest during Survey 8 (1.84%; CI: 0.38 - 5.28) and not detected during Survey 7.

Among the participants who were not vaccinated in KE Kombewa, no clear trend was noted for 3D7 haplotype prevalence. Other haplotypes showed low prevalence, with a few exceptions such as CIG32 with comparatively higher prevalence of 11.97% (Survey 6).

Figure 9.3 Haplotype prevalence in KE Kombewa over survey - Unvaccinated participants - Analysis Set



KE = Kenya.
Source: Figure 14.8.2.10 (08APR2025 23:44 GMT)

Vaccinated participants:

Survey 6: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most prevalent haplotype was CIG33 (11.11%). 3D7 haplotype was not detected (Table 14.4.3.10).

Survey 7: Among all detected haplotypes, the most prevalent haplotypes were CIG32, CIG33, and CIG39 (4.48% each), individually. No detected haplotypes were present in >5% of the participants. 3D7 haplotype was not detected (Table 14.5.3.10).

Survey 8: No detected haplotypes had a prevalence of >5%. The most prevalent haplotypes were CIG33 and CIG39 (3.35% prevalence each), where 8 other haplotypes showed prevalence of >1%. Additionally, 3D7 haplotype was not detected (Table 9.20).

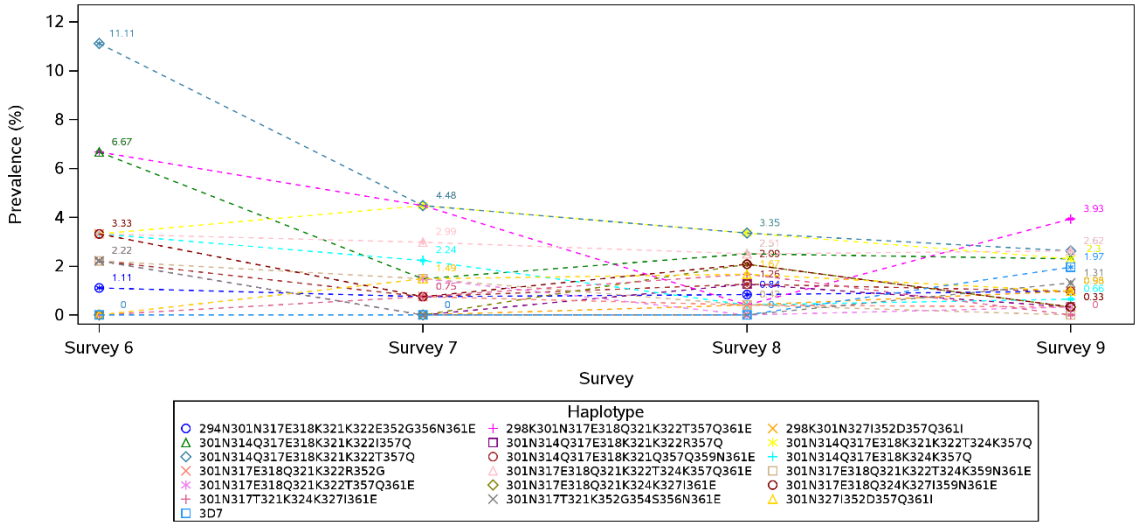
Survey 9: No detected haplotypes had a prevalence of >5%. The most prevalent haplotype was CIG32 (3.93%) as compared to 1.97% for 3D7 haplotype. Five other haplotypes showed frequency of >1% (Table 9.21).

Among the participants who received RTS,S/AS01$_E$ vaccination, 3D7 haplotype was not detected in KE Kombewa during Surveys 6, 7, and 8. Additionally, prevalence was low during Survey 9 (1.97%; CI: 0.73 - 4.23).

Among vaccinated participants in KE Kombewa, CIG33 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG32 was most prevalent during Surveys 7 and 9.

The haplotypes did not show any clear trend in prevalence across surveys in the overall or unvaccinated populations; however, for vaccinated participants: CIG33 showed a quick decrease in prevalence from Survey 6 (11.11%) through Survey 9 (2.62%).

Figure 9.4 Haplotype prevalence in KE Kombewa over survey - Vaccinated participants - Analysis Set



KE = Kenya.
Source: Figure 14.8.2.9 (08APR2025 23:44 GMT)

Table 9.20 Haplotype prevalence by site according to RTS,S/AS01$_E$ vaccination status - Analysis Set, Survey 8

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Unvac	3D7	0	142	0.00	0	2.56
		CIG11	1	142	0.70	0.02	3.86
		CIG31	1	142	0.70	0.02	3.86
		CIG32	5	142	3.52	1.15	8.03
		CIG39	1	142	0.70	0.02	3.86

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG33	3	142	2.11	0.44	6.05
		CIG313	1	142	0.70	0.02	3.86
		CIG34	1	142	0.70	0.02	3.86
		CIG18	1	142	0.70	0.02	3.86
		CIG35	3	142	2.11	0.44	6.05
		CIG37	2	142	1.41	0.17	5
	Vac	3D7	0	256	0.00	0	1.43
		CIG32	7	256	2.73	1.11	5.55
		CIG15	1	256	0.39	0.01	2.16
		CIG16	1	256	0.39	0.01	2.16
		CIG17	1	256	0.39	0.01	2.16
		CIG114	1	256	0.39	0.01	2.16
		CIG34	2	256	0.78	0.09	2.79
		CIG12	2	256	0.78	0.09	2.79
		CIG35	2	256	0.78	0.09	2.79
		CIG36	2	256	0.78	0.09	2.79
		CIG13	2	256	0.78	0.09	2.79
KE_Kombewa	Unvac	3D7	3	163	1.84	0.38	5.28
		CIG311	2	163	1.23	0.15	4.36
		CIG31	2	163	1.23	0.15	4.36
		CIG32	9	163	5.52	2.56	10.22
		CIG38	8	163	4.91	2.14	9.44
		CIG39	7	163	4.29	1.74	8.65
		CIG33	4	163	2.45	0.67	6.16
		CIG34	4	163	2.45	0.67	6.16
		CIG35	4	163	2.45	0.67	6.16
		CIG36	4	163	2.45	0.67	6.16
		CIG37	4	163	2.45	0.67	6.16
	Vac	3D7	0	239	0.00	0	1.53
		CIG38	6	239	2.51	0.93	5.38
		CIG312	3	239	1.26	0.26	3.62
		CIG39	8	239	3.35	1.46	6.49
		CIG33	8	239	3.35	1.46	6.49
		CIG310	3	239	1.26	0.26	3.62
		CIG35	6	239	2.51	0.93	5.38
		CIG36	5	239	2.09	0.68	4.81
		CIG21	5	239	2.09	0.68	4.81
		CIG37	4	239	1.67	0.46	4.23
		CIG22	4	239	1.67	0.46	4.23

CI = confidence interval; GH = Ghana; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; Unvac=unvaccinated; Vac=vaccinated.

n = number of participants infected with the specific *P. falciparum* haplotype

N = total number of enrolled participants

% = n/N × 100

LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 15 reads is considered.

The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.

Source: Table 14.6.3.10 (08APR2025 23:42 GMT)

Table 9.21 Haplotype prevalence by site according to RTS,S/AS01$_E$ vaccination status - Analysis Set, Survey 9

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Unvac	3D7	1	100	1.00	0.03	5.45
		CIG315	1	100	1.00	0.03	5.45
		CIG111	1	100	1.00	0.03	5.45
		CIG112	1	100	1.00	0.03	5.45
		CIG311	1	100	1.00	0.03	5.45
		CIG32	1	100	1.00	0.03	5.45
		CIG113	1	100	1.00	0.03	5.45
		CIG38	2	100	2.00	0.24	7.04
		CIG120	1	100	1.00	0.03	5.45
		CIG310	2	100	2.00	0.24	7.04
		CIG34	2	100	2.00	0.24	7.04
	Vac	3D7	0	300	0.00	0	1.22
		CIG315	1	300	0.33	0.01	1.84
		CIG311	1	300	0.33	0.01	1.84
		CIG31	1	300	0.33	0.01	1.84
		CIG32	2	300	0.67	0.08	2.39
		CIG38	3	300	1.00	0.21	2.89
		CIG312	3	300	1.00	0.21	2.89
		CIG39	2	300	0.67	0.08	2.39
		CIG33	3	300	1.00	0.21	2.89
		CIG310	3	300	1.00	0.21	2.89
		CIG119	2	300	0.67	0.08	2.39
KE_Kombewa	Unvac	3D7	1	96	1.04	0.03	5.67
		CIG32	3	96	3.13	0.65	8.86
		CIG312	3	96	3.13	0.65	8.86
		CIG39	6	96	6.25	2.33	13.11
		CIG33	7	96	7.29	2.98	14.45
		CIG313	2	96	2.08	0.25	7.32
		CIG34	2	96	2.08	0.25	7.32
		CIG35	4	96	4.17	1.15	10.33
		CIG36	3	96	3.13	0.65	8.86
		CIG37	2	96	2.08	0.25	7.32
		CIG22	3	96	3.13	0.65	8.86
	Vac	3D7	6	305	1.97	0.73	4.23
		CIG315	3	305	0.98	0.2	2.85
		CIG32	12	305	3.93	2.05	6.77
		CIG23	3	305	0.98	0.2	2.85
		CIG38	7	305	2.30	0.93	4.67
		CIG39	7	305	2.30	0.93	4.67
		CIG33	8	305	2.62	1.14	5.1
		CIG310	3	305	0.98	0.2	2.85
		CIG35	8	305	2.62	1.14	5.1
		CIG24	4	305	1.31	0.36	3.32
		CIG22	3	305	0.98	0.2	2.85

CI = confidence interval; GH = Ghana; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; Unvac=unvaccinated; Vac=vaccinated

n = number of participants infected with the specific *P. falciparum* haplotype

N = total number of enrolled participants

% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.3.10 (08APR2025 23:42 GMT)

9.4.2. Longitudinal estimates of *P. falciparum* haplotype frequency by age group, gender and RTS,S/AS01$_E$ vaccination status

For participants who were infected with specific *P. falciparum* haplotypes, the frequency of detected *P. falciparum* haplotypes by site, age group, gender and by vaccination status is described below.

9.4.2.1. Site-wise frequency estimation of *P. falciparum* haplotypes by gender

For participants who were infected with the specific *P. falciparum* haplotypes, the frequency of detected *P. falciparum* haplotypes by site and gender i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Survey 3 (Table 14.1.2.3), Survey 4 (Table 14.2.2.3), Survey 5 (Table 14.3.2.3), Survey 6 (Table 14.4.2.3), and Survey 7 (Table 14.5.2.3) is presented in the IR dated 26 November 2024. The frequency of *P. falciparum* haplotypes by site and according to gender i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Surveys 8 and 9 is provided in Table 14.6.2.3 (presented in Table 9.22) and in Table 14.7.2.3 (presented in Table 9.23), respectively.

A graphical representation of the frequency of *P. falciparum* haplotypes by site and according to gender, is provided in Figure 14.6.2.4 and Figure 14.7.2.4 for Survey 8 and Survey 9, respectively.

GH Kintampo:

Female participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were 3D7 and CIG38 (8.67% each) (Table 14.1.2.3).

Survey 4: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG32 and CIG38, each of which had a frequency of 8.21% as compared to 6.72% for 3D7 (Table 14.2.2.3).

Survey 5: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 10.45% as compared to 7.46% for 3D7 (Table 14.3.2.3).

Survey 6: Among all detected haplotypes, CIG32 and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 8.06% as compared to 4.03% for 3D7 (Table 14.4.2.3).

Survey 7: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG33, CIG38, CIG39, CIG122, and CIG314 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG32 and CIG21, each had a frequency of 10.53% as compared to 7.89% for 3D7 haplotype (Table 14.5.2.3).

Survey 8: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG37 were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (16.23%). 3D7 haplotype was not detected (Table 9.22).

Survey 9: Among all detected haplotypes, CIG14, CIG32, CIG33, and CIG310 were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG32 and CIG310 (8.57%, each) as compared to 2.86% for 3D7 haplotype (detected in 1 female participant) (Table 9.23).

Among female participants in GH Kintampo, CIG32 was the most frequently detected haplotype across all surveys except for Survey 3. The frequency of 3D7 haplotype was highest in Survey 3 (8.67%) and was not detected among female participants during Survey 8.

Male participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 with a frequency of 9.47% as compared to 6.32% for 3D7 (Table 14.1.2.3).

Survey 4: Among all detected haplotypes, 3D7, CIG32, CIG33, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 13.75% as compared to 8.75% for 3D7 (Table 14.2.2.3).

Survey 5: Among all detected haplotypes, 3D7, CIG12, CIG31, CIG32, CIG33, CIG38, CIG39, CIG121, CIG125, and CIG314 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was 3D7 (9.26%) (Table 14.3.2.3).

Survey 6: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG36, CIG38, and CIG122 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 13.68% as compared to 5.26% for 3D7 (Table 14.4.2.3).

Survey 7: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, CIG39, and CIG312 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were 3D7, CIG32, and CIG33 (10.64% each) (Table 14.5.2.3).

Survey 8: Among all detected haplotypes, CIG32 and CIG36 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (26.81%). Additionally, haplotype 3D7 was not detected (Table 9.22).

Survey 9: Among all detected haplotypes, CIG33, CIG34, CIG38, CIG39, CIG310, and CIG312 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG38 (11.11%). Additionally, haplotype 3D7 was not detected (Table 9.23).

Among male participants in GH Kintampo, CIG32 was the most frequently detected haplotype across Surveys 4, 6, 7, and 8. The frequency of 3D7 haplotype was highest in Survey 7 (10.64%) and was not detected among male participants during Surveys 8 and 9.

KE Kombewa:

Female participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 8.81% as compared to 2.07% for 3D7 (Table 14.1.2.3).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG36, CIG38, CIG39, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 11.67% as compared to 0.56% for 3D7 (Table 14.2.2.3).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 with a frequency of 14.62% as compared to 1.54% for 3D7 (Table 14.3.2.3).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35 and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 14.14% as compared to 0.51% for 3D7 (Table 14.4.2.3).

Survey 7: Among all detected haplotypes, CIG21, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG32 and CIG33 (9.52% each). 3D7 haplotype was not detected (Table 14.5.2.3).

Survey 8: Among all detected haplotypes, CIG38 and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG38 (8.40%) as compared with 0.76% for 3D7 haplotype (detected in 1 female participant) (Table 9.22).

Survey 9: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG39 were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (11.25%) as compared with 3.75% for 3D7 haplotype (Table 9.23).

Among female participants in KE Kombewa, CIG32 was the most frequently detected haplotype during Surveys 3, 4, 6, and 7. The frequency of 3D7 haplotype was highest in Survey 9 (3.75%) and was not detected among female participants during Survey 7.

Male participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (11.90%). 3D7 haplotype was not detected (Table 14.1.2.3).

Survey 4. Among all detected haplotypes, CIG32, CIG33, CIG35 and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (13.04%). 3D7 haplotype was not detected (Table 14.2.2.3).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35 and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 12.57% as compared to 1.20% for 3D7 (Table 14.3.2.3).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38 and CIG312 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 with a frequency of 11.88% as compared to 0.63% for 3D7 haplotype (Table 14.4.2.3).

Survey 7: Among all detected haplotypes, CIG22, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (10.71%). 3D7 haplotype was not detected (Table 14.5.2.3).

Survey 8: Among all detected haplotypes, CIG21, CIG32, CIG33, CIG35, CIG36, CIG37, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 (10.67%) as compared to 2.67% for 3D7 haplotype (detected in 2 male participants) (Table 9.22).

Survey 9: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (10.23%) as compared to 4.55% for 3D7 haplotype (detected in 4 male participants) (Table 9.23).

Among male participants in KE Kombewa, CIG32 was the most frequently detected haplotype during Surveys 3, 4, 5, and 9. The frequency of 3D7 haplotype was highest in Survey 9 (4.55%) and was not detected among male participants during Surveys 3, 4, and 7.

Table 9.22 Haplotype frequency by site according to gender- Analysis Set, Survey 8

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Female	3D7	0	43	0.00	0	8.22
		CIG32	7	43	16.28	6.81	30.7
		CIG33	4	43	9.30	2.59	22.14
		CIG35	4	43	9.30	2.59	22.14
		CIG37	3	43	6.98	1.46	19.06
		CIG34	2	43	4.65	0.57	15.81
		CIG31	1	43	2.33	0.06	12.29
		CIG15	1	43	2.33	0.06	12.29
		CIG16	1	43	2.33	0.06	12.29
		CIG114	1	43	2.33	0.06	12.29
		CIG116	1	43	2.33	0.06	12.29
	Male	3D7	0	21	0.00	0	16.11
		CIG32	5	21	23.81	8.22	47.17
		CIG36	2	21	9.52	1.17	30.38
		CIG11	1	21	4.76	0.12	23.82
		CIG17	1	21	4.76	0.12	23.82
		CIG34	1	21	4.76	0.12	23.82
		CIG18	1	21	4.76	0.12	23.82
		CIG19	1	21	4.76	0.12	23.82
		CIG110	1	21	4.76	0.12	23.82
		CIG12	1	21	4.76	0.12	23.82
		CIG35	1	21	4.76	0.12	23.82
KE_Kombewa	Female	3D7	1	131	0.76	0.02	4.18
		CIG38	11	131	8.40	4.27	14.53
		CIG39	7	131	5.34	2.18	10.7
		CIG33	6	131	4.58	1.7	9.7
		CIG32	5	131	3.82	1.25	8.68
		CIG36	5	131	3.82	1.25	8.68
		CIG311	4	131	3.05	0.84	7.63
		CIG310	4	131	3.05	0.84	7.63
		CIG34	4	131	3.05	0.84	7.63
		CIG35	4	131	3.05	0.84	7.63
		CIG26	4	131	3.05	0.84	7.63
	Male	3D7	2	75	2.67	0.32	9.3
		CIG39	8	75	10.67	4.72	19.94
		CIG33	7	75	9.33	3.84	18.29
		CIG35	6	75	8.00	2.99	16.6
		CIG21	6	75	8.00	2.99	16.6
		CIG32	5	75	6.67	2.2	14.88
		CIG37	5	75	6.67	2.2	14.88
		CIG36	4	75	5.33	1.47	13.1
		CIG38	3	75	4.00	0.83	11.25
		CIG315	2	75	2.67	0.32	9.3
		CIG31	2	75	2.67	0.32	9.3

CI = confidence interval; GH = Ghana; KE = Kenya
n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.2.3 (08APR2025 23:38 GMT)

Table 9.23 Haplotype frequency by site according to gender- Analysis Set, Survey 9

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Female	3D7	1	35	2.86	0.07	14.92
		CIG32	3	35	8.57	1.8	23.06
		CIG310	3	35	8.57	1.8	23.06
		CIG33	2	35	5.71	0.7	19.16
		CIG14	2	35	5.71	0.7	19.16
		CIG315	1	35	2.86	0.07	14.92
		CIG111	1	35	2.86	0.07	14.92
		CIG112	1	35	2.86	0.07	14.92
		CIG311	1	35	2.86	0.07	14.92
		CIG31	1	35	2.86	0.07	14.92
		CIG113	1	35	2.86	0.07	14.92
	Male	3D7	0	36	0.00	0	9.74
		CIG38	4	36	11.11	3.11	26.06
		CIG34	3	36	8.33	1.75	22.47
		CIG312	2	36	5.56	0.68	18.66
		CIG39	2	36	5.56	0.68	18.66
		CIG33	2	36	5.56	0.68	18.66
		CIG310	2	36	5.56	0.68	18.66
		CIG315	1	36	2.78	0.07	14.53
		CIG311	1	36	2.78	0.07	14.53
		CIG118	1	36	2.78	0.07	14.53
		CIG314	1	36	2.78	0.07	14.53
KE_Kombewa	Female	3D7	3	80	3.75	0.78	10.57
		CIG33	9	80	11.25	5.28	20.28
		CIG39	7	80	8.75	3.59	17.2
		CIG32	6	80	7.50	2.8	15.61
		CIG35	6	80	7.50	2.8	15.61
		CIG34	3	80	3.75	0.78	10.57
		CIG36	3	80	3.75	0.78	10.57
		CIG24	3	80	3.75	0.78	10.57
		CIG22	3	80	3.75	0.78	10.57
		CIG315	2	80	2.50	0.3	8.74
		CIG313	2	80	2.50	0.3	8.74
	Male	3D7	4	88	4.55	1.25	11.23
		CIG32	9	88	10.23	4.78	18.53
		CIG38	7	88	7.95	3.26	15.7
		CIG39	6	88	6.82	2.54	14.25
		CIG33	6	88	6.82	2.54	14.25
		CIG35	6	88	6.82	2.54	14.25
		CIG23	3	88	3.41	0.71	9.64
		CIG312	3	88	3.41	0.71	9.64
		CIG24	3	88	3.41	0.71	9.64
		CIG22	3	88	3.41	0.71	9.64
		CIG315	2	88	2.27	0.28	7.97

CI = confidence interval; GH = Ghana; KE = Kenya

n = number of participants infected with the specific *P. falciparum* haplotype
N = total number of enrolled participants
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.2.3 (08APR2025 23:38 GMT)

9.4.2.2. Site-wise frequency estimation of *P. falciparum* haplotypes by age group

For participants who were infected with specific *P. falciparum* haplotypes, the frequency of detected *P. falciparum* haplotypes by site and age group i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Survey 3 (Table 14.1.2.5), Survey 4 (Table 14.2.2.5), Survey 5 (Table 14.3.2.5), Survey 6 (Table 14.4.2.5), and Survey 7 (Table 14.5.2.5) is presented in the IR dated 26 November 2024. The frequency of *P. falciparum* haplotypes by site and according to age group i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Surveys 8 and 9 is provided in Table 14.6.2.5 (presented in Table 9.24) and in Table 14.7.2.5 (presented in Table 9.25), respectively.

A graphical representation of the frequency of *P. falciparum* haplotypes by site and according to age group, is provided in Figure 14.6.2.6 and Figure 14.7.2.6 for Survey 8 and Survey 9, respectively.

GH Kintampo:

0.5 to 1 year old participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 with a frequency of 10.23% as compared to 7.95% for 3D7 haplotype (Table 14.1.2.5).

Survey 4: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG38, and CIG312 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 10.09% as compared to 6.42% for 3D7 haplotype (Table 14.2.2.5).

Survey 5: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 with a frequency of 14.81% as compared to 7.41% for 3D7 haplotype (Table 14.3.2.5).

Survey 6: Among all detected haplotypes, CIG32 and CIG33 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 11.49% as compared to 4.60% for 3D7 haplotype (Table 14.4.2.5).

Survey 7: All detected haplotypes were present in >5% of the participants. Among all detected haplotypes, the most frequently detected haplotypes were CIG32 and CIG312 (16.67% each). 3D7 haplotype was not detected (Table 14.5.2.5).

Survey 8: Among all detected haplotypes, CIG13 and CIG32 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (17.86%), while 3D7 haplotype was not detected (Table 9.24).

Survey 9: The most frequently detected haplotypes were CIG32, CIG33, CIG34, CIG39, CIG119, and CIG310 (6.90%, each) while no other haplotype showed frequency of >5%. Additionally, 3D7 haplotype was not detected (Table 9.25).

Among 0.5 to 1 year old participants in GH Kintampo, CIG32 was the most frequently detected haplotype across all surveys except for Surveys 3 and 5. The frequency of 3D7 haplotype was highest during Survey 3 (7.95%) and not detected during Surveys 7, 8, and 9.

2 to 4 years old participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 with a frequency of 8.33% as compared to 7.14% for 3D7 haplotype (Table 14.1.2.5).

Survey 4: Among all detected haplotypes, 3D7, CIG32, CIG33, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 11.89% as compared to 8.65% for 3D7 haplotype (Table 14.2.2.5).

Survey 5: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 9.57% as compared to 8.51% for 3D7 haplotype (Table 14.3.2.5).

Survey 6: Among all detected haplotypes, CIG32, CIG38 and CIG122 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 9.85% as compared to 4.55% for 3D7 haplotype (Table 14.4.2.5).

Survey 7: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was 3D7 (11.94%) (Table 14.5.2.5).

Survey 8: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG36, and CIG37 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (19.44%), while 3D7 haplotype was not detected (Table 9.24).

Survey 9: Among all detected haplotypes, CIG38, CIG310, and CIG312 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG38 (9.52%) as compared to 2.38% for 3D7 haplotype (detected in 1 participant from the 2 to 4 years old group) (Table 9.25).

Among 2 to 4 years old participants in GH Kintampo, CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8. The 3D7 haplotype frequency was highest during Survey 7 (11.94%) and not detected during Survey 8.

KE Kombewa:

0.5 to 1 year old participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 11.76% as compared to 0.74% for 3D7 haplotype (Table 14.1.2.5).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG37, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 13.45% as compared to 0.84% for 3D7 haplotype (Table 14.2.2.5).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG38, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG32 and CIG38, each of which had a frequency of 10.43% as compared to 0.87% for 3D7 haplotype (Table 14.3.2.5).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (12.41%). 3D7 haplotype was not detected (Table 14.4.2.5).

Survey 7: Among all detected haplotypes, CIG22, CIG32, CIG33, CIG34, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (11.25%). 3D7 haplotype was not detected (Table 14.5.2.5).

Survey 8: Among all detected haplotypes, CIG21, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG35 and CIG39 (6.98%, each) as compared to 1.16% for 3D7 haplotype (detected in 1 participant) (Table 9.24).

Survey 9: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (10.61%) as compared to 7.58% for 3D7 haplotype (Table 9.25).

Among 0.5 to 1 year old participants in KE Kombewa, CIG32 was the most frequent during Surveys 3, 4, 5, 7, and 9. 3D7 haplotype frequency was highest in Survey 9 (7.58%) and was not detected in Surveys 6 and 7.

2 to 4 years old participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 9.33% as compared to 1.33% for 3D7 haplotype (Table 14.1.2.5).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, CIG38, CIG39, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (11.84%). 3D7 haplotype was not detected (Table 14.2.2.5).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38 and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 with a frequency of 13.19% as compared to 1.65% for 3D7 haplotype (Table 14.3.2.5).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG38 and CIG35 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 14.08% as compared to 0.94% for 3D7 haplotype (Table 14.4.2.5).

Survey 7: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (10.30%). 3D7 haplotype was not detected (Table 14.5.2.5).

Survey 8: Among all detected haplotypes, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG38, CIG39, and CIG33 (7.50%, each) as compared to 1.67% for 3D7 haplotype (detected in 2 participants) (Table 9.24).

Survey 9: Among all detected haplotypes, CIG22, CIG32, CIG33, CIG35, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (9.80%) as compared to 1.96% for 3D7 haplotype (detected in 2 participants) (Table 9.25).

Among 2 to 4 years old participants in KE Kombewa, CIG32 was the most frequently detected haplotype across Surveys 3, 4, and 6, while CIG33 was the most frequently detected haplotype across Surveys 5, 7, 8, and 9. The frequency of 3D7 haplotype was highest in Survey 9 (1.96%) and was not detected in Surveys 4 and 7.

Table 9.24 Haplotype frequency by site according to age group- Analysis Set, Survey 8

						95% CI	
Site	Age Group	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	0.5-1Y	3D7	0	28	0.00	0	12.34
		CIG32	5	28	17.86	6.06	36.89
		CIG13	2	28	7.14	0.88	23.5
		CIG15	1	28	3.57	0.09	18.35
		CIG16	1	28	3.57	0.09	18.35

						95% CI	
Site	**Age Group**	**Haplotype**	**n**	**N**	**Proportion (in %)**	**LL**	**UL**
		CIG114	1	28	3.57	0.09	18.35
		CIG116	1	28	3.57	0.09	18.35
		CIG115	1	28	3.57	0.09	18.35
		CIG117	1	28	3.57	0.09	18.35
		CIG314	1	28	3.57	0.09	18.35
		CIG33	1	28	3.57	0.09	18.35
	2-4Y	3D7	0	36	0.00	0	9.74
		CIG32	7	36	19.44	8.19	36.02
		CIG35	4	36	11.11	3.11	26.06
		CIG33	3	36	8.33	1.75	22.47
		CIG34	2	36	5.56	0.68	18.66
		CIG36	2	36	5.56	0.68	18.66
		CIG37	2	36	5.56	0.68	18.66
		CIG11	1	36	2.78	0.07	14.53
		CIG31	1	36	2.78	0.07	14.53
		CIG17	1	36	2.78	0.07	14.53
		CIG38	1	36	2.78	0.07	14.53
KE_Kombewa	0.5-1Y	3D7	1	86	1.16	0.03	6.31
		CIG39	6	86	6.98	2.6	14.57
		CIG35	6	86	6.98	2.6	14.57
		CIG38	5	86	5.81	1.91	13.05
		CIG21	5	86	5.81	1.91	13.05
		CIG33	4	86	4.65	1.28	11.48
		CIG36	4	86	4.65	1.28	11.48
		CIG37	4	86	4.65	1.28	11.48
		CIG26	3	86	3.49	0.73	9.86
		CIG22	3	86	3.49	0.73	9.86
		CIG32	2	86	2.33	0.28	8.15
	2-4Y	3D7	2	120	1.67	0.2	5.89
		CIG38	9	120	7.50	3.49	13.76
		CIG39	9	120	7.50	3.49	13.76
		CIG33	9	120	7.50	3.49	13.76
		CIG32	8	120	6.67	2.92	12.71
		CIG36	5	120	4.17	1.37	9.46
		CIG35	4	120	3.33	0.92	8.31
		CIG37	4	120	3.33	0.92	8.31
		CIG315	3	120	2.50	0.52	7.13
		CIG311	3	120	2.50	0.52	7.13
		CIG310	3	120	2.50	0.52	7.13

CI = confidence interval; GH = Ghana; KE = Kenya; WHO = World Health Organisation.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date; IC = Informed consent
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population in a given category
% = n/N × 100
LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.6.2.5 (08APR2025 23:38 GMT)

Table 9.25 Haplotype frequency by site according to age group - Analysis Set, Survey 9

						95% CI	
Site	Gender	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	0.5-1Y	3D7	0	29	0.00	0	11.94
		CIG32	2	29	6.90	0.85	22.77
		CIG39	2	29	6.90	0.85	22.77
		CIG33	2	29	6.90	0.85	22.77
		CIG310	2	29	6.90	0.85	22.77
		CIG34	2	29	6.90	0.85	22.77
		CIG119	2	29	6.90	0.85	22.77
		CIG315	1	29	3.45	0.09	17.76
		CIG31	1	29	3.45	0.09	17.76
		CIG118	1	29	3.45	0.09	17.76
		CIG314	1	29	3.45	0.09	17.76
	2-4Y	3D7	1	42	2.38	0.06	12.57
		CIG38	4	42	9.52	2.66	22.62
		CIG312	3	42	7.14	1.5	19.48
		CIG310	3	42	7.14	1.5	19.48
		CIG311	2	42	4.76	0.58	16.16
		CIG33	2	42	4.76	0.58	16.16
		CIG35	2	42	4.76	0.58	16.16
		CIG315	1	42	2.38	0.06	12.57
		CIG111	1	42	2.38	0.06	12.57
		CIG112	1	42	2.38	0.06	12.57
		CIG32	1	42	2.38	0.06	12.57
KE_Kombewa	0.5-1Y	3D7	5	66	7.58	2.51	16.8
		CIG32	7	66	10.61	4.37	20.64
		CIG38	6	66	9.09	3.41	18.74
		CIG39	5	66	7.58	2.51	16.8
		CIG33	5	66	7.58	2.51	16.8
		CIG35	5	66	7.58	2.51	16.8
		CIG23	2	66	3.03	0.37	10.52
		CIG314	2	66	3.03	0.37	10.52
		CIG27	2	66	3.03	0.37	10.52
		CIG36	2	66	3.03	0.37	10.52
		CIG25	2	66	3.03	0.37	10.52
	2-4Y	3D7	2	102	1.96	0.24	6.9
		CIG33	10	102	9.80	4.8	17.29
		CIG32	8	102	7.84	3.45	14.87
		CIG39	8	102	7.84	3.45	14.87
		CIG35	7	102	6.86	2.8	13.63
		CIG22	6	102	5.88	2.19	12.36
		CIG24	5	102	4.90	1.61	11.07
		CIG315	4	102	3.92	1.08	9.74
		CIG312	4	102	3.92	1.08	9.74
		CIG310	4	102	3.92	1.08	9.74
		CIG34	3	102	2.94	0.61	8.36

CI = confidence interval; GH = Ghana; KE = Kenya; WHO = World Health Organisation.
WHO age categories: 0.5-1Y = 6 months to 1 year at IC date, 2-4Y = 2 to 4 years at IC date; IC = Informed consent
n = number of occurrences of a specific haplotype in the population
N = total number of haplotypes detected in the population in a given category
% = n/N × 100

LL, UL = 95% Lower and Upper exact confidence limits
The threshold of 15 reads is considered.
The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.
Source: Table 14.7.2.5 (08APR2025 23:38 GMT)

9.4.2.3. Site-wise frequency estimation of *P. falciparum* haplotypes by vaccination status

For participants who were infected with the specific *P. falciparum* haplotypes, the frequency of detected *P. falciparum* haplotypes by site and RTS,S/AS01$_E$ vaccination status i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Survey 3 (Table 14.1.2.7), Survey 4 (Table 14.2.2.7), Survey 5 (Table 14.3.2.7), Survey 6 (Table 14.4.2.7), and Survey 7 (Table 14.5.2.7) is presented in the IR dated 26 November 2024. The frequency of *P. falciparum* haplotypes by site and according to RTS,S/AS01$_E$ vaccination status i.e., the 10 most frequently detected haplotypes along with those having ≥5% frequency during Surveys 8 and 9 is provided in Table 14.6.2.7 (presented in Table 9.26) and in Table 14.7.2.7 (presented in Table 9.27), respectively.

A graphical representation of the frequency of *P. falciparum* haplotypes by site and according to RTS,S/AS01$_E$ vaccination status, is provided in Figure 14.6.2.8 and Figure 14.7.2.8 for Survey 8 and Survey 9, respectively.

GH Kintampo:

Unvaccinated participants:

Survey 3: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG39 with a frequency of 8.82% as compared to 7.35% for 3D7 (Table 14.1.2.7).

Survey 4: Among all detected haplotypes, 3D7, CIG32, CIG33, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 11.22% as compared to 7.82% for 3D7 (Table 14.2.2.7).

Survey 5: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 9.09% as compared to 8.26% for 3D7 (Table 14.3.2.7).

Survey 6: Among all detected haplotypes, CIG32, CIG38 and CIG122 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 10.28% as compared to 4.67% for 3D7 (Table 14.4.2.7).

Survey 7: Among all detected haplotypes, 3D7, CIG21, CIG32, CIG33, CIG39, and CIG122 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was 3D7 (10.67%) (Table 14.5.2.7).

Survey 8: Among all detected haplotypes, CIG32, CIG33, and CIG35 haplotypes were present in >10% of the participants and CIG37 was present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (20.83%), while 3D7 haplotype was not detected (Table 9.26).

Survey 9: Among all detected haplotypes, CIG34, CIG38, and CIG310haplotypes were present in >5% of the participants, individually. The most frequently detected haplotypes were CIG34, CIG38, and CIG310 (6.25% each) as compared to 3.13% for 3D7 haplotype (detected in 1 participant) (Table 9.27).

Among unvaccinated participants in GH Kintampo, CIG32 was the most frequently detected haplotype across Surveys 4, 5, 6, and 8. The frequency of 3D7 haplotype was highest during Survey 7 (10.67%) and not detected among unvaccinated participants during Survey 8.

Vaccinated participants:

Survey 6: Among all detected haplotypes, CIG12, CIG32, CIG39, CIG126, and CIG127 haplotypes were detected in 20% of the participants. 3D7 haplotype was not detected (Table 14.4.2.7).

Survey 7: Among all detected haplotypes, CIG14, CIG17, CIG32, CIG33, CIG37, CIG38, CIG311, and CIG312, haplotypes were detected in >5% of participants, individually. The most frequently detected haplotypes were CIG32 and CIG38 (20% each). 3D7 haplotype was not detected (Table 14.5.2.7).

Survey 8: Among all detected haplotypes, CIG12, CIG13, CIG32, CIG34, CIG35, and CIG36 haplotypes were detected in ≥5% of participants, individually. The most frequently detected haplotype was CIG32 (17.50%), while 3D7 haplotype was not detected (Table 9.26).

Survey 9: Among all detected haplotypes, CIG32, CIG33, CIG38, CIG39, CIG119,CIG310, and CIG312 haplotypes were detected in >5% of participants, individually. The most frequently detected haplotypes were CIG33, CIG38, CIG310, and CIG312 (7.69%, each), while 3D7 haplotype was not detected (Table 9.27).

Among vaccinated participants in GH Kintampo, CIG32 was the most frequently detected haplotype across Surveys 7 and 8, while CIG38 was most frequently detected across Surveys 7 and 9. The 3D7 haplotype was not detected among vaccinated participants during Surveys 6, 7, 8, and 9.

KE Kombewa

Unvaccinated participants:

Survey 3: Among all detected haplotypes, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 10.25% as compared to 1.11% for 3D7 (Table 14.1.2.7).

Survey 4: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, CIG39, and CIG310 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 12.36% as compared to 0.27% for 3D7 (Table 14.2.2.7).

Survey 5: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 with a frequency of 11.78% as compared to 1.35% for 3D7 (Table 14.3.2.7).

Survey 6: Among all detected haplotypes, CIG32, CIG33, CIG35 and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 with a frequency of 12.98% as compared to 0.70% for 3D7 (Table 14.4.2.7).

Survey 7: Among all detected haplotypes, CIG21, CIG32, CIG33, CIG35, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (9.33%). 3D7 haplotype was not detected (Table 14.5.2.7).

Survey 8: Among all detected haplotypes, CIG32, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (9.47%) as compared to 3.16% for 3D7 haplotype (Table 9.26).

Survey 9: Among all detected haplotypes, CIG33, CIG35, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (9.86%) as compared to 1.41% for 3D7 haplotype (detected in 1 participant) (Table 9.27), while all others had frequency of >1%.

Among unvaccinated participants in KE Kombewa, CIG32 was the most frequently detected haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most frequently detected during Surveys 5, 7, and 9. The frequency of 3D7 haplotype was highest during Survey 8 (3.16%) and not detected during Survey 7.

Vaccinated participants:

Survey 6: Among all detected haplotypes, CIG32, CIG33, and CIG38 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (13.70%). 3D7 haplotype was not detected (Table 14.4.2.7).

Survey 7: Among all detected haplotypes, CIG32, CIG33, CIG34, CIG35, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (13.46%). 3D7 haplotype was not detected (Table 14.5.2.7).

Survey 8: Among all detected haplotypes, CIG21, CIG33, CIG35, CIG38, and CIG39 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG33 (8.11%), while 3D7 haplotype was not detected (Table 9.26).

Survey 9: Among all detected haplotypes, CIG32, CIG33, CIG35, CIG38, CIG39, and CIG24 haplotypes were present in >5% of the participants, individually. The most frequently detected haplotype was CIG32 (12.37%) as compared to 6.19% for 3D7 haplotype (Table 9.27).

Among vaccinated participants in KE Kombewa, CIG33 was the most frequently detected haplotype across Surveys 6, 7, and 8. The frequency of 3D7 haplotype was 6.19% during Survey 9, but was not detected during Surveys 6, 7, and 8.

Table 9.26 Haplotype frequency by site according to RTS,S/AS01$_E$ vaccination status - Analysis Set, Survey 8

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Unvac	3D7	0	24	0.00	0	14.25
		CIG32	5	24	20.83	7.13	42.15
		CIG33	3	24	12.50	2.66	32.36
		CIG35	3	24	12.50	2.66	32.36
		CIG37	2	24	8.33	1.03	27
		CIG11	1	24	4.17	0.11	21.12
		CIG31	1	24	4.17	0.11	21.12
		CIG39	1	24	4.17	0.11	21.12
		CIG313	1	24	4.17	0.11	21.12
		CIG34	1	24	4.17	0.11	21.12
		CIG18	1	24	4.17	0.11	21.12
	Vac	3D7	0	40	0.00	0	8.81
		CIG32	7	40	17.50	7.34	32.78
		CIG34	2	40	5.00	0.61	16.92
		CIG12	2	40	5.00	0.61	16.92
		CIG35	2	40	5.00	0.61	16.92
		CIG36	2	40	5.00	0.61	16.92
		CIG13	2	40	5.00	0.61	16.92
		CIG15	1	40	2.50	0.06	13.16
		CIG16	1	40	2.50	0.06	13.16
		CIG17	1	40	2.50	0.06	13.16
		CIG114	1	40	2.50	0.06	13.16
KE_Kombewa	Unvac	3D7	3	95	3.16	0.66	8.95
		CIG32	9	95	9.47	4.42	17.22
		CIG38	8	95	8.42	3.71	15.92
		CIG39	7	95	7.37	3.01	14.59
		CIG33	4	95	4.21	1.16	10.43
		CIG34	4	95	4.21	1.16	10.43
		CIG35	4	95	4.21	1.16	10.43
		CIG36	4	95	4.21	1.16	10.43
		CIG37	4	95	4.21	1.16	10.43
		CIG311	2	95	2.11	0.26	7.4
		CIG31	2	95	2.11	0.26	7.4
	Vac	3D7	0	111	0.00	0	3.27
		CIG33	9	111	8.11	3.77	14.83
		CIG39	8	111	7.21	3.16	13.71
		CIG38	6	111	5.41	2.01	11.39
		CIG35	6	111	5.41	2.01	11.39
		CIG21	6	111	5.41	2.01	11.39

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG36	5	111	4.50	1.48	10.2
		CIG37	4	111	3.60	0.99	8.97
		CIG22	4	111	3.60	0.99	8.97
		CIG312	3	111	2.70	0.56	7.7
		CIG310	3	111	2.70	0.56	7.7

CI = confidence interval; GH = Ghana; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; Unvac=unvaccinated; Vac=vaccinated

n = number of occurrences of a specific haplotype in the population

N = total number of haplotypes detected in the population in a given category

% = n/N × 100

LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 15 reads is considered.

The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.

Source: Table 14.6.2.7 (08APR2025 23:38 GMT)

Table 9.27 Haplotype frequency by site according to RTS,S/AS01$_E$ vaccination status - Analysis Set, Survey 9

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
GH_Kintampo	Unvac	3D7	1	32	3.13	0.08	16.22
		CIG38	2	32	6.25	0.77	20.81
		CIG310	2	32	6.25	0.77	20.81
		CIG34	2	32	6.25	0.77	20.81
		CIG315	1	32	3.13	0.08	16.22
		CIG111	1	32	3.13	0.08	16.22
		CIG112	1	32	3.13	0.08	16.22
		CIG311	1	32	3.13	0.08	16.22
		CIG32	1	32	3.13	0.08	16.22
		CIG113	1	32	3.13	0.08	16.22
		CIG120	1	32	3.13	0.08	16.22
	Vac	3D7	0	39	0.00	0	9.03
		CIG38	3	39	7.69	1.62	20.87
		CIG312	3	39	7.69	1.62	20.87
		CIG33	3	39	7.69	1.62	20.87
		CIG310	3	39	7.69	1.62	20.87
		CIG32	2	39	5.13	0.63	17.32
		CIG39	2	39	5.13	0.63	17.32
		CIG119	2	39	5.13	0.63	17.32
		CIG315	1	39	2.56	0.06	13.48
		CIG311	1	39	2.56	0.06	13.48
		CIG31	1	39	2.56	0.06	13.48
KE_Kombewa	Unvac	3D7	1	71	1.41	0.04	7.6
		CIG33	7	71	9.86	4.06	19.26
		CIG39	6	71	8.45	3.16	17.49
		CIG35	4	71	5.63	1.56	13.8
		CIG32	3	71	4.23	0.88	11.86
		CIG312	3	71	4.23	0.88	11.86
		CIG36	3	71	4.23	0.88	11.86
		CIG22	3	71	4.23	0.88	11.86
		CIG313	2	71	2.82	0.34	9.81

						95% CI	
Site	Status	Haplotype	n	N	Proportion (in %)	LL	UL
		CIG34	2	71	2.82	0.34	9.81
		CIG37	2	71	2.82	0.34	9.81
	Vac	3D7	6	97	6.19	2.3	12.98
		CIG32	12	97	12.37	6.56	20.61
		CIG33	8	97	8.25	3.63	15.61
		CIG35	8	97	8.25	3.63	15.61
		CIG38	7	97	7.22	2.95	14.3
		CIG39	7	97	7.22	2.95	14.3
		CIG24	5	97	5.15	1.69	11.62
		CIG315	3	97	3.09	0.64	8.77
		CIG23	3	97	3.09	0.64	8.77
		CIG310	3	97	3.09	0.64	8.77
		CIG22	3	97	3.09	0.64	8.77

CI = confidence interval; GH = Ghana; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome; Unvac=unvaccinated; Vac=vaccinated

n = number of occurrences of a specific haplotype in the population

N = total number of haplotypes detected in the population in a given category

% = n/N × 100

LL, UL = 95% Lower and Upper exact confidence limits

The threshold of 15 reads is considered.

The 3D7 haplotype and the haplotypes with a frequency greater or equal to 5% in the site during the survey with at least the 10 most frequent haplotypes detected are presented.

Source: Table 14.7.2.7 (08APR2025 23:39 GMT)

9.4.3. Site-wise trends in longitudinal prevalence of specific *P. falciparum* haplotypes with and without RTS,S/AS01$_E$ vaccination

Trends analysis was performed for 3D7 haplotype and haplotypes with ≥5% frequency across all 7 surveys, by site and according to RTS,S/AS01$_E$ vaccination status.

Longitudinal analyses of site-wise prevalence of infection with the following P. falciparum haplotypes are described below:

- 3D7 haplotype
- CIG32 haplotype
- CIG33 haplotype
- CIG35 haplotype (Kombewa only)
- CIG38 haplotype
- CIG39 haplotype

The reference survey is the earliest survey where the haplotype is detected.

9.4.3.1. Infection with *Plasmodium falciparum* 3D7 haplotype

9.4.3.1.1. Trends in 3D7 haplotype infection using univariable logistic regression

GH Kintampo

The crude OR to define the relationship between *P. falciparum* 3D7 haplotype infection and survey year in GH Kintampo using univariable logistic regression is provided for overall population in Table 14.8.1.6 (presented in Table 9.28), for vaccinated participants in Table 14.8.1.7, and for unvaccinated participants in Table 14.8.1.8 (presented in Table 9.29).

The crude OR to define the relationship between P. falciparum 3D7 haplotype infection and survey year in GH Kintampo using univariable piecewise logistic regression is provided in Table 14.8.1.9 (using fixed breakpoint of Survey 7) and in Table 14.8.1.10 (using model-identified breakpoint).

In the overall population in GH Kintampo there was a downward trend for OR of P. falciparum 3D7 haplotype infection starting from Survey 5, with significant reduction in OR (unadjusted) noted by Survey 9 compared to Survey 3 (Table 9.28). In the unvaccinated population, there was a non-significant trend towards a decreased risk estimated (Table 9.29).

The analysis could not be performed for the vaccinated population in GH Kintampo as the 3D7 haplotype infection was not detected in the vaccinated population (Table 14.8.1.7).

A drop in OR estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.721 and post-breakpoint OR = 0.434) in GH Kintampo, while model-identified breakpoint was estimated at Survey 7.

Table 9.28 Univariable logistic regression on survey associated with 3D7 haplotype's infection in GH Kintampo - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	23	5.8	0.922	0.514	1.654
	Survey 5	399	10	2.5	0.387	0.183	0.816
	Survey 6	402	10	2.5	0.384	0.182	0.810
	Survey 7	399	8	2.0	0.308	0.137	0.691
	Survey 8	398	0	0	-	-	-
	Survey 9	400	1	0.3	0.038	0.005	0.280

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.6 (08APR2025 23:40 GMT)

Table 9.29 Univariable logistic regression on survey associated with 3D7 haplotype's infection in GH Kintampo - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	23	5.8	0.922	0.514	1.654
	Survey 5	399	10	2.5	0.387	0.183	0.816
	Survey 6	356	10	2.8	0.435	0.206	0.918
	Survey 7	241	8	3.3	0.516	0.229	1.164
	Survey 8	142	0	0	-	-	-
	Survey 9	100	1	1.0	0.152	0.020	1.135

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.8 (08APR2025 23:40 GMT)

KE Kombewa

The crude OR to define the relationship between *P. falciparum* 3D7 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.1 (presented in Table 9.30), for unvaccinated participants in Table 14.8.1.3 (presented in Table 9.31), and for vaccinated participants in Table 14.8.1.2 (presented in Table 9.32).

The crude OR to define the relationship between P. falciparum 3D7 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.4 (with fixed breakpoint of Survey 7) and in Table 14.8.1.5 (with model-identified breakpoint).

No significant OR was estimated on surveys in KE Kombewa in the overall population (Table 9.30) as well as the unvaccinated population (Table 9.31). The 3D7 haplotype infection was not detected in the vaccinated population in KE Kombewa during Surveys 6, 7, and 8 (Table 9.32).

No significant OR was estimated using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.705 and post-breakpoint OR = 1.664) in KE Kombewa, while model-identified breakpoint was estimated at Survey 7.

Table 9.30 Univariable logistic regression on survey associated with 3D7 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	4	1.0	Reference	-	-
	Survey 4	398	1	0.3	0.249	0.028	2.241
	Survey 5	402	4	1.0	0.995	0.247	4.006
	Survey 6	399	2	0.5	0.499	0.091	2.738

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
	Survey 7	400	0	0	-	-	-
	Survey 8	402	3	0.7	0.744	0.166	3.347
	Survey 9	401	7	1.7	1.759	0.511	6.056

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.1 (08APR2025 23:40 GMT)

Table 9.31 Univariable logistic regression on survey associated with 3D7 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	4	1.0	Reference	-	-
	Survey 4	398	1	0.3	0.249	0.028	2.241
	Survey 5	402	4	1.0	0.995	0.247	4.006
	Survey 6	309	2	0.6	0.645	0.117	3.544
	Survey 7	266	0	0	-	-	-
	Survey 8	163	3	1.8	1.856	0.411	8.387
	Survey 9	96	1	1.0	1.042	0.115	9.431

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.3 (08APR2025 23:40 GMT)

Table 9.32 Univariable logistic regression on survey associated with 3D7 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	0	0	-	-	-
	Survey 7	134	0	0	-	-	-
	Survey 8	239	0	0	-	-	-
	Survey 9	305	6	2.0	Reference	-	-

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.2 (08APR2025 23:41 GMT)

9.4.3.1.2. Trends in 3D7 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status

GH Kintampo

The adjusted OR of *P. falciparum* 3D7 haplotype infection in GH Kintampo using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.14 (presented in Table 9.33).

The adjusted OR of *P. falciparum* 3D7 haplotype infection in GH Kintampo using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.15 (using fixed breakpoint of Survey 7) and in Table 14.8.1.16 (using model-identified breakpoint).

No significant OR (adjusted) was estimated in GH Kintampo for surveys compared with Survey 3, as well as comparisons between genders, age group, and multiple infections. A significant OR adjusted for multiple infection (5.128; CI: 2.74 – 9.60) was noted for prevalence of *P. falciparum* 3D7 haplotype infection in GH Kintampo. The OR adjusted for RTS,S/AS01$_E$ vaccination status could not be calculated (Table 9.33).

No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.867 and post-breakpoint OR = 0.845; Table 14.8.1.15) in GH Kintampo, while model-identified breakpoint was estimated at Survey 7 (Table 14.8.1.16).

Table 9.33 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with 3D7 haplotype's infection in GH Kintampo - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	23	5.8	0.893	0.463	1.724
	Survey 5	399	10	2.5	0.817	0.352	1.897
	Survey 6	402	10	2.5	0.526	0.231	1.198
	Survey 7	399	8	2.0	1.066	0.420	2.704
	Survey 8	398	0	0	-	-	-
	Survey 9	400	1	0.3	0.375	0.043	3.250
Gender	Female	1373	36	2.6	Reference	-	-
	Male	1424	41	2.9	0.871	0.519	1.461
Age group	0.5-1Y	1257	20	1.6	Reference	-	-
	2-4Y	1540	57	3.7	1.451	0.812	2.592
RTS,S/AS01 vaccination status	Unvaccinated	2037	77	3.8	-	-	-
	Vaccinated	760	0	0	Reference	-	-

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Multiple infection	Children infected with >1 haplotype	267	63	23.6	5.128	2.740	9.597
	Children infected with 1 haplotype	243	14	5.8	Reference	-	-
	Children not infected	2287	0	0	-	-	-

CI = confidence interval; GH = Ghana; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.14 (08APR2025 23:39 GMT)

KE Kombewa

The adjusted OR of *P. falciparum* 3D7 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.11 (presented in Table 9.34).

The adjusted OR of *P. falciparum* 3D7 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.12 (using fixed breakpoint of Survey 7) and in Table 14.8.1.13 (using model-identified breakpoint).

3D7 haplotype was not detected during Survey 7. No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3, except for Survey 9 (4.73; CI: 1.05 – 21.24). A significant OR adjusted for multiple infection (3.57; CI: 1.27 - 10.05) was noted (Table 9.34).

A significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 for the post-breakpoint group (OR = 2.169; CI: 1.26 – 3.73) (Table 14.8.1.12) in KE Kombewa, while model-identified breakpoint was estimated at Survey 7 (Table 14.8.1.13).

Table 9.34 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with 3D7 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	4	1.0	Reference	-	-
	Survey 4	398	1	0.3	0.239	0.026	2.172
	Survey 5	402	4	1.0	1.228	0.298	5.062

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
	Survey 6	399	2	0.5	0.587	0.104	3.322
	Survey 7	400	0	0	-	-	-
	Survey 8	402	3	0.7	1.524	0.285	8.165
	Survey 9	401	7	1.7	4.726	1.051	21.240
Gender	Female	1445	12	0.8	Reference	-	-
	Male	1357	9	0.7	0.714	0.289	1.763
Age group	0.5-1Y	1268	9	0.7	Reference	-	-
	2-4Y	1534	12	0.8	0.846	0.328	2.179
RTS,S/AS01 vaccination status	Unvaccinated	2034	15	0.7	1.245	0.331	4.687
	Vaccinated	768	6	0.8	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	16	3.7	3.572	1.270	10.046
	Children infected with 1 haplotype	375	5	1.3	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.11 (08APR2025 23:39 GMT)

9.4.3.2. Infection with *P. falciparum* CIG32 haplotype

9.4.3.2.1. Trends in CIG32 haplotype infection using univariable logistic regression

GH Kintampo

The crude OR to define the relationship between *P. falciparum* CIG32 haplotype infection and survey year in GH Kintampo using univariable logistic regression is provided for overall population in Table 14.8.1.22 (presented in Table 9.35), for unvaccinated participants in Table 14.8.1.24 (presented in Table 9.36), and for vaccinated participants in Table 14.8.1.23 (presented in Table 9.37).

The crude OR to define the relationship between *P. falciparum* CIG32 haplotype infection and survey year in GH Kintampo using univariable piecewise logistic regression is provided in Table 14.8.1.25 (fixed breakpoint of Survey 7) and in Table 14.8.1.26 (model-identified breakpoint).

In the overall population in GH Kintampo a significant OR of *P. falciparum* CIG32 haplotype infection was detected at Surveys 7 and 9 compared to Survey 3 (Table 9.35). No significant OR was obtained on unvaccinated or vaccinated populations in GH Kintampo (Table 9.36 and Table 9.37, respectively).

A significant OR was observed using fixed breakpoint of Survey 7 on post-breakpoint group OR = 0.627 (CI: 0.477 - 0.823) on *P. falciparum* CIG32 haplotype infection in GH Kintampo, while model-identified breakpoint was estimated at Survey 8.

Table 9.35 Univariable logistic regression on survey associated with CIG32 haplotype's infection in GH Kintampo - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	22	5.5	Reference	-	-
	Survey 4	398	33	8.3	1.558	0.891	2.722
	Survey 5	399	11	2.8	0.488	0.234	1.021
	Survey 6	402	23	5.7	1.045	0.573	1.908
	Survey 7	399	9	2.3	0.398	0.181	0.874
	Survey 8	398	12	3.0	0.536	0.261	1.098
	Survey 9	400	3	0.8	0.130	0.039	0.439

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.22 (08APR2025 23:40 GMT)

Table 9.36 Univariable logistic regression on survey associated with CIG32 haplotype's infection in GH Kintampo - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	22	5.5	Reference	-	-
	Survey 4	398	33	8.3	1.558	0.891	2.722
	Survey 5	399	11	2.8	0.488	0.234	1.021
	Survey 6	356	22	6.2	1.135	0.617	2.086
	Survey 7	241	7	2.9	0.515	0.217	1.225
	Survey 8	142	5	3.5	0.629	0.234	1.693
	Survey 9	100	1	1.0	0.174	0.023	1.307

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.24 (08APR2025 23:40 GMT)

Table 9.37 Univariable logistic regression on survey associated with CIG32 haplotype's infection in GH Kintampo - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	46	1	2.2	Reference	-	-
	Survey 7	158	2	1.3	0.577	0.051	6.509
	Survey 8	256	7	2.7	1.265	0.152	10.531
	Survey 9	300	2	0.7	0.302	0.027	3.399

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.23 (08APR2025 23:40 GMT)

KE Kombewa

The crude OR to define the relationship between *P. falciparum* CIG32 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.17 (presented in Table 9.38), for unvaccinated participants in Table 14.8.1.19 (presented in Table 9.39), and for vaccinated participants in Table 14.8.1.18 (presented in Table 9.40).

The crude OR to define the relationship between *P. falciparum* CIG32 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.20 (fixed breakpoint of Survey 7) and in Table 14.8.1.21 (model-identified breakpoint).

Significant OR of *P. falciparum* CIG32 haplotype infection were detected at Surveys 7, 8 and 9 (OR = 0.571, OR = 0.250, and OR = 0.381 respectively) compared to Survey 3 in KE Kombewa in the overall population. In the vaccinated population, a significant OR (0.059; CI: 0.007 - 0.496) was noted only at Survey 8 (Table 9.40), while no significant OR was estimated for the unvaccinated population (Table 9.39).

A significant OR was observed using fixed breakpoint of Survey 7 on post-breakpoint OR = 0.635 (CI: 0.523 - 0.771) on *P. falciparum* CIG32 haplotype infection in KE Kombewa (Table 14.8.1.20). A similar observation was made when model identified the breakpoint with a breakpoint estimate to Survey 6 (Table 14.8.1.21).

Table 9.38 Univariable logistic regression on survey associated with CIG32 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	37	9.3	Reference	-	-
	Survey 4	398	45	11.3	1.251	0.790	1.979

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
	Survey 5	402	31	7.7	0.820	0.498	1.350
	Survey 6	399	43	10.8	1.185	0.746	1.883
	Survey 7	400	22	5.5	0.571	0.330	0.987
	Survey 8	402	10	2.5	0.250	0.123	0.511
	Survey 9	401	15	3.7	0.381	0.206	0.706

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.17 (08APR2025 23:40 GMT)

Table 9.39 Univariable logistic regression on survey associated with CIG32 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	37	9.3	Reference	-	-
	Survey 4	398	45	11.3	1.251	0.790	1.979
	Survey 5	402	31	7.7	0.820	0.498	1.350
	Survey 6	309	37	12.0	1.335	0.824	2.161
	Survey 7	266	16	6.0	0.628	0.342	1.153
	Survey 8	163	9	5.5	0.573	0.270	1.217
	Survey 9	96	3	3.1	0.316	0.095	1.049

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.19 (08APR2025 23:40 GMT)

Table 9.40 Univariable logistic regression on survey associated with CIG32 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	6	6.7	Reference	-	-
	Survey 7	134	6	4.5	0.656	0.205	2.103
	Survey 8	239	1	0.4	0.059	0.007	0.496
	Survey 9	305	12	3.9	0.573	0.209	1.574

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.18 (08APR2025 23:41 GMT)

9.4.3.2.2. Trends in CIG32 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status

GH Kintampo

The adjusted OR of *P. falciparum* CIG32 haplotype infection in GH Kintampo using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.30 (presented in Table 9.41).

The adjusted OR of *P. falciparum* CIG32 haplotype infection in GH Kintampo using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.31 (fixed breakpoint of Survey 7) and in Table 14.8.1.32 (model-identified breakpoint).

No significant OR (adjusted) was estimated in GH Kintampo for surveys compared with Survey 3. A significant OR adjusted for multiple infection (4.77; CI: 2.85 - 7.98) was noted for prevalence of *P. falciparum* CIG32 haplotype infection in GH Kintampo (Table 9.41). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 1.177 and post-breakpoint OR = 0.744; Table 14.8.1.31) in GH Kintampo, while model-identified breakpoint was estimated at Survey 8 (Table 14.8.1.32).

Table 9.41 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG32 haplotype's infection in GH Kintampo - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	22	5.5	Reference	-	-
	Survey 4	398	33	8.3	1.728	0.916	3.259
	Survey 5	399	11	2.8	1.130	0.494	2.587
	Survey 6	402	23	5.7	1.813	0.903	3.640
	Survey 7	399	9	2.3	1.204	0.485	2.990
	Survey 8	398	12	3.0	3.154	1.024	9.715
	Survey 9	400	3	0.8	0.353	0.077	1.631
Gender	Female	1373	53	3.9	Reference	-	-
	Male	1424	60	4.2	1.060	0.676	1.664
Age group	0.5-1Y	1257	39	3.1	Reference	-	-
	2-4Y	1540	74	4.8	1.073	0.656	1.754
RTS,S/AS01 vaccination status	Unvaccinated	2037	101	5.0	0.530	0.175	1.610
	Vaccinated	760	12	1.6	Reference	-	-

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Multiple infection	Children infected with >1 haplotype	267	89	33.3	4.765	2.846	7.977
	Children infected with 1 haplotype	243	24	9.9	Reference	-	-
	Children not infected	2287	0	0	-	-	-

CI = confidence interval; GH = Ghana; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.30 (08APR2025 23:39 GMT)

KE Kombewa

The adjusted OR of *P. falciparum* CIG32 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.27 (presented in Table 9.42).

The adjusted OR of *P. falciparum* CIG32 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.28 (fixed breakpoint of Survey 7) and in Table 14.8.1.29 (model-identified breakpoint).

No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3. A significant OR adjusted for multiple infection (4.15; CI: 2.85 - 6.05) was noted for prevalence of *P. falciparum* CIG32 haplotype infection in KE Kombewa (Table 9.42). A significant OR was observed using fixed breakpoint of Survey 7 on the post-breakpoint group = 0.754 (CI: 0.592 - 0.961) (Table 14.8.1.28), while the model-identified breakpoint was estimated at Survey 6 with a significant OR on the post-breakpoint group = 0.754 (CI: 0.577-0.986) (Table 14.8.1.29).

Table 9.42 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG32 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Survey	Survey 3	400	37	9.3	Reference	-	-
	Survey 4	398	45	11.3	1.300	0.760	2.225
	Survey 5	402	31	7.7	0.985	0.555	1.750
	Survey 6	399	43	10.8	1.611	0.922	2.817
	Survey 7	400	22	5.5	0.778	0.411	1.472

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
	Survey 8	402	10	2.5	0.458	0.198	1.060
	Survey 9	401	15	3.7	0.884	0.397	1.965
Gender	Female	1445	97	6.7	Reference	-	-
	Male	1357	106	7.8	1.102	0.786	1.545
Age group	0.5-1Y	1268	74	5.8	Reference	-	-
	2-4Y	1534	129	8.4	1.126	0.781	1.624
RTS,S/AS01 vaccination status	Unvaccinated	2034	178	8.8	1.210	0.648	2.259
	Vaccinated	768	25	3.3	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	159	36.4	4.154	2.854	6.047
	Children infected with 1 haplotype	375	44	11.7	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG32 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.27 (08APR2025 23:39 GMT)

9.4.3.3. Infection with *P. falciparum* CIG33 haplotype

9.4.3.3.1. Trends in CIG33 haplotype infection using univariable logistic regression

GH Kintampo

The crude OR to define the relationship between *P. falciparum* CIG33 haplotype infection and survey year in GH Kintampo using univariable logistic regression is provided for overall population in Table 14.8.1.70 (presented in Table 9.43), for unvaccinated participants in Table 14.8.1.72 (presented in Table 9.44), and for vaccinated participants in Table 14.8.1.71 (presented in Table 9.45).

The crude OR to define the relationship between *P. falciparum* CIG33 haplotype infection and survey year in GH Kintampo using univariable piecewise logistic regression is provided in Table 14.8.1.73 (fixed breakpoint of Survey 7) and in Table 14.8.1.74 (model-identified breakpoint).

A significant OR of *P. falciparum* CIG33 haplotype infection was detected at Survey 5 through 9, compared to Survey 3 (Table 9.43). A significant OR = 0.276 (CI: 0.110-0.692) was detected at Survey 5 in the unvaccinated population (Table 9.44), while no significant OR was observed in the vaccinated population (Table 9.45).

Significant OR was observed using fixed breakpoint of Survey 7 on the pre-breakpoint group = 0.722 (CI: 0.571-0.912) (Table 14.8.1.73). Similarly, significant OR was observed using model-identified breakpoint, estimated at Survey 7, on pre-breakpoint group = 0.742 (CI: 0.644-0.854) (Table 14.8.1.74).

Table 9.43 Univariable logistic regression on survey associated with CIG33 haplotype's infection in GH Kintampo - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	21	5.2	Reference	-	-
	Survey 4	398	18	4.5	0.857	0.450	1.634
	Survey 5	399	6	1.5	0.276	0.110	0.692
	Survey 6	402	10	2.5	0.462	0.215	0.993
	Survey 7	399	8	2.0	0.370	0.162	0.846
	Survey 8	398	4	1.0	0.184	0.062	0.540
	Survey 9	400	4	1.0	0.183	0.062	0.537

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.70 (08APR2025 23:40 GMT)

Table 9.44 Univariable logistic regression on survey associated with CIG33 haplotype's infection in GH Kintampo - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	21	5.2	Reference	-	-
	Survey 4	398	18	4.5	0.857	0.450	1.634
	Survey 5	399	6	1.5	0.276	0.110	0.692
	Survey 6	356	10	2.8	0.523	0.243	1.126
	Survey 7	241	7	2.9	0.541	0.227	1.293
	Survey 8	142	3	2.1	0.391	0.115	1.330
	Survey 9	100	1	1.0	0.183	0.024	1.375

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.72 (08APR2025 23:40 GMT)

Table 9.45 Univariable logistic regression on survey associated with CIG33 haplotype's infection in GH Kintampo - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	46	0	0	-	-	-
	Survey 7	158	1	0.6	Reference	-	-
	Survey 8	256	1	0.4	0.616	0.038	9.914
	Survey 9	300	3	1.0	1.586	0.164	15.372

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.71 (08APR2025 23:40 GMT)

KE Kombewa

The crude OR to define the relationship between *P. falciparum* CIG33 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.65 (presented in Table 9.46), for unvaccinated participants in Table 14.8.1.67 (presented in Table 9.47), and for vaccinated participants in Table 14.8.1.66 (presented in Table 9.48).

The crude OR to define the relationship between *P. falciparum* CIG33 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.68 (fixed breakpoint of Survey 7) and in Table 14.8.1.69 (model-identified breakpoint).

A significant OR for *P. falciparum* CIG33 haplotype infection was noted during Surveys 8 and 9 in the overall population (Table 9.46), during Survey 8 in the unvaccinated population (Table 9.47), and during Surveys 8 and 9 in the vaccinated populations (Table 9.48).

Table 9.46 Univariable logistic regression on survey associated with CIG33 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	30	7.5	Reference	-	-
	Survey 4	398	38	9.5	1.302	0.789	2.147
	Survey 5	402	34	8.5	1.139	0.683	1.901
	Survey 6	399	41	10.3	1.412	0.863	2.312
	Survey 7	400	24	6.0	0.787	0.452	1.372
	Survey 8	402	12	3.0	0.380	0.192	0.753
	Survey 9	401	15	3.7	0.479	0.254	0.905

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection

% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.65 (08APR2025 23:40 GMT)

Table 9.47 Univariable logistic regression on survey associated with CIG33 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	30	7.5	Reference	-	-
	Survey 4	398	38	9.5	1.302	0.789	2.147
	Survey 5	402	34	8.5	1.139	0.683	1.901
	Survey 6	309	31	10.0	1.375	0.813	2.326
	Survey 7	266	18	6.8	0.895	0.488	1.641
	Survey 8	163	4	2.5	0.310	0.108	0.895
	Survey 9	96	7	7.3	0.970	0.413	2.280

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.67 (08APR2025 23:41 GMT)

Table 9.48 Univariable logistic regression on survey associated with CIG33 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	10	11.1	Reference	-	-
	Survey 7	134	6	4.5	0.375	0.131	1.072
	Survey 8	239	8	3.3	0.277	0.106	0.726
	Survey 9	305	8	2.6	0.215	0.082	0.564

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.66 (08APR2025 23:41 GMT)

9.4.3.3.2. Trends in CIG33 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status

GH Kintampo

The adjusted OR of *P. falciparum* CIG33 haplotype infection in GH Kintampo using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.78 (presented in Table 9.49).

The adjusted OR of *P. falciparum* CIG33 haplotype infection in GH Kintampo using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.79 (fixed breakpoint of Survey 7) and in Table 14.8.1.80 (model-identified breakpoint).

No significant OR (adjusted) was estimated for surveys compared with Survey 3. A significant OR adjusted for multiple infection (5.0; CI: 2.62 - 9.52) was noted for prevalence of *P. falciparum* CIG33 haplotype infection in GH Kintampo (Table 9.49). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.895 and post-breakpoint OR = 1.236; Table 14.8.1.79), while model-identified breakpoint was estimated at Survey 5 (Table 14.8.1.80).

Table 9.49 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG33 haplotype's infection in GH Kintampo - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Survey	Survey 3	401	21	5.2	Reference	-	-
	Survey 4	398	18	4.5	0.812	0.400	1.647
	Survey 5	399	6	1.5	0.575	0.214	1.548
	Survey 6	402	10	2.5	0.655	0.284	1.510
	Survey 7	399	8	2.0	1.244	0.485	3.194
	Survey 8	398	4	1.0	1.024	0.262	4.009
	Survey 9	400	4	1.0	0.958	0.232	3.961
Gender	Female	1373	32	2.3	Reference	-	-
	Male	1424	39	2.7	1.034	0.611	1.750
Age group	0.5-1Y	1257	22	1.8	Reference	-	-
	2-4Y	1540	49	3.2	1.121	0.628	2.001
RTS,S/AS01 vaccination status	Unvaccinated	2037	66	3.2	1.298	0.340	4.950
	Vaccinated	760	5	0.7	Reference	-	-
Multiple infection	Children infected with >1 haplotype	267	58	21.7	4.997	2.623	9.516
	Children infected with 1 haplotype	243	13	5.3	Reference	-	-
	Children not infected	2287	0	0	-	-	-

CI = confidence interval; GH = Ghana; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.78 (08APR2025 23:39 GMT)

KE Kombewa

The adjusted OR of *P. falciparum* CIG33 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.75 (presented in Table 9.50).

The adjusted OR of *P. falciparum* CIG33 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.76 (fixed breakpoint of Survey 7) and in Table 14.8.1.77 (model-identified breakpoint).

No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3. A significant OR adjusted for multiple infection (3.32; CI: 2.3 - 4.8) was noted for prevalence of *P. falciparum* CIG33 haplotype infection in KE Kombewa (Table 9.50). Significant OR was observed using fixed breakpoint of Survey 7 on the post-breakpoint group = 0.703 (CI: 0.553 - 0.893), while model-identified breakpoint was estimated at Survey 6, with a significant change in OR (adjusted) observed on the post-breakpoint group = 0.714 (CI: 0.557 - 0.915) (Table 14.8.1.77).

Table 9.50 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG33 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Survey	Survey 3	400	30	7.5	Reference	-	-
	Survey 4	398	38	9.5	1.352	0.770	2.375
	Survey 5	402	34	8.5	1.506	0.843	2.689
	Survey 6	399	41	10.3	1.781	1.001	3.168
	Survey 7	400	24	6.0	1.052	0.553	2.001
	Survey 8	402	12	3.0	0.545	0.239	1.244
	Survey 9	401	15	3.7	0.820	0.363	1.854
Gender	Female	1445	96	6.6	Reference	-	-
	Male	1357	98	7.2	0.977	0.697	1.370
Age group	0.5-1Y	1268	72	5.7	Reference	-	-
	2-4Y	1534	122	8.0	1.208	0.837	1.745
RTS,S/AS01 vaccination status	Unvaccinated	2034	162	8.0	0.692	0.380	1.259
	Vaccinated	768	32	4.2	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	146	33.4	3.317	2.297	4.790
	Children infected with 1 haplotype	375	48	12.8	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG33 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.75 (08APR2025 23:39 GMT)

9.4.3.4. Infection with *Plasmodium falciparum* CIG35 haplotype

9.4.3.4.1. Trends in CIG35 haplotype infection using univariable logistic regression

GH Kintampo

The *P. falciparum* CIG35 haplotype was not detected with ≥5% prevalence across all surveys. Hence univariable logistic regression analysis with this haplotype was not performed for the site in GH Kintampo.

KE Kombewa

The crude OR to define the relationship between *P. falciparum* CIG35 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.81 (presented in Table 9.51), for unvaccinated participants in Table 14.8.1.83 (presented in Table 9.52), and for vaccinated participants in Table 14.8.1.82 (presented in Table 9.53).

The crude OR to define the relationship between *P. falciparum* CIG35 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.84 (fixed breakpoint of Survey 7) and in Table 14.8.1.85 (model-identified breakpoint).

No significant OR was estimated on surveys in the overall, unvaccinated, or vaccinated populations in KE Kombewa.

A significant OR was observed using fixed breakpoint of Survey 7 on the post-breakpoint group = 0.699 (CI: 0.563 - 0.867) (Table 14.8.1.84), while the model-identified breakpoint was estimated at Survey 6 with a significant OR on the post-breakpoint group = 0.701 (CI: 0.556 - 0.884) (Table 14.8.1.85).

Table 9.51 Univariable logistic regression on survey associated with CIG35 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	19	4.8	Reference	-	-
	Survey 4	398	29	7.3	1.576	0.868	2.860
	Survey 5	402	22	5.5	1.161	0.618	2.180

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
	Survey 6	399	28	7.0	1.513	0.831	2.757
	Survey 7	400	16	4.0	0.836	0.423	1.649
	Survey 8	402	10	2.5	0.512	0.235	1.114
	Survey 9	401	12	3.0	0.619	0.296	1.292

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG35 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.81 (08APR2025 23:40 GMT)

Table 9.52 Univariable logistic regression on survey associated with CIG35 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	19	4.8	Reference	-	-
	Survey 4	398	29	7.3	1.576	0.868	2.860
	Survey 5	402	22	5.5	1.161	0.618	2.180
	Survey 6	309	25	8.1	1.765	0.953	3.268
	Survey 7	266	12	4.5	0.947	0.452	1.986
	Survey 8	163	4	2.5	0.505	0.169	1.507
	Survey 9	96	4	4.2	0.872	0.290	2.624

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG35 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.83 (08APR2025 23:41 GMT)

Table 9.53 Univariable logistic regression on survey associated with CIG35 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	3	3.3	Reference	-	-
	Survey 7	134	4	3.0	0.892	0.195	4.084
	Survey 8	239	6	2.5	0.747	0.183	3.051
	Survey 9	305	8	2.6	0.781	0.203	3.007

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG35 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.82 (08APR2025 23:41 GMT)

9.4.3.4.2. Trends in CIG35 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status

GH Kintampo

The *P. falciparum* CIG35 haplotype was not detected with ≥5% prevalence across all surveys. Hence logistic regression analysis with this haplotype was not performed for the site in GH Kintampo.

KE Kombewa

The adjusted OR of *P. falciparum* CIG35 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.86 (presented in Table 9.54).

The adjusted OR of *P. falciparum* CIG35 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.87 (fixed breakpoint of Survey 7) and in Table 14.8.1.88 (model-identified breakpoint).

No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3. A significant OR adjusted for multiple infection (3.17; CI: 2.07 - 4.85) was noted for prevalence of *P. falciparum* CIG35 haplotype infection in KE Kombewa (Table 9.54). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 1.154 and post-breakpoint OR = 0.856; Table 14.8.1.87) in KE Kombewa, while model-identified breakpoint was estimated at Survey 6 (Table 14.8.1.88).

Table 9.54 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG35 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	19	4.8	Reference	-	-
	Survey 4	398	29	7.3	1.655	0.869	3.149
	Survey 5	402	22	5.5	1.448	0.734	2.857
	Survey 6	399	28	7.0	1.980	1.021	3.840
	Survey 7	400	16	4.0	1.241	0.588	2.619
	Survey 8	402	10	2.5	1.031	0.419	2.533
	Survey 9	401	12	3.0	1.498	0.608	3.687
Gender	Female	1445	64	4.4	Reference	-	-
	Male	1357	72	5.3	1.116	0.763	1.634

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Age group	0.5-1Y	1268	56	4.4	Reference	-	-
	2-4Y	1534	80	5.2	0.873	0.581	1.314
RTS,S/AS01 vaccination status	Unvaccinated	2034	115	5.7	1.169	0.599	2.282
	Vaccinated	768	21	2.7	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	103	23.6	3.167	2.070	4.845
	Children infected with 1 haplotype	375	33	8.8	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with 3D7 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.86 (08APR2025 23:39 GMT)

9.4.3.5. Infection with *Plasmodium falciparum* CIG38 haplotype

9.4.3.5.1. Trends in CIG38 haplotype infection using univariable logistic regression

GH Kintampo

The crude OR to define the relationship between *P. falciparum* CIG38 haplotype infection and survey year in GH Kintampo using univariable logistic regression is provided for overall population in Table 14.8.1.38 (presented in Table 9.55), for unvaccinated participants in Table 14.8.1.40 (presented in Table 9.56), and for vaccinated participants in Table 14.8.1.39 (presented in Table 9.57).

The crude OR to define the relationship between *P. falciparum* 3D7 haplotype infection and survey year in GH Kintampo using univariable piecewise logistic regression is provided in Table 14.8.1.41 (fixed breakpoint of Survey 7) and in Table 14.8.1.42 (model-identified breakpoint).

In the overall population in GH Kintampo a significant OR of *P. falciparum* CIG38 haplotype infection was detected at Survey 5 through 9, compared to Survey 3 (Table 9.55).

P. falciparum CIG38 haplotype was not detected in the unvaccinated population in GH Kintampo during Survey 8. A significant OR of *P. falciparum* CIG38 haplotype infection was observed at Survey 5 and Survey 7 in the unvaccinated population (Table 9.56). No significant OR was obtained for the vaccinated population (Table 9.57).

Significant OR was observed using fixed breakpoint of Survey 7 on pre-breakpoint group = 0.695 (CI: 0.555 - 0.872) and post-breakpoint group = 0.680 (CI: 0.470 - 0.984) (Table 14.8.1.41). Similarly, significant OR was observed using model-identified breakpoint, estimated at Survey 8, on pre-breakpoint group = 0.647 (CI: 0.550 - 0.761) (Table 14.8.1.42).

Table 9.55 Univariable logistic regression on survey associated with CIG38 haplotype's infection in GH Kintampo - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	18	4.5	0.712	0.382	1.328
	Survey 5	399	9	2.3	0.347	0.160	0.753
	Survey 6	402	12	3.0	0.463	0.229	0.934
	Survey 7	399	5	1.3	0.191	0.072	0.504
	Survey 8	398	1	0.3	0.038	0.005	0.281
	Survey 9	400	5	1.3	0.190	0.072	0.502

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.38 (08APR2025 23:40 GMT)

Table 9.56 Univariable logistic regression on survey associated with CIG38 haplotype's infection in GH Kintampo - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	18	4.5	0.712	0.382	1.328
	Survey 5	399	9	2.3	0.347	0.160	0.753
	Survey 6	356	12	3.4	0.525	0.260	1.060
	Survey 7	241	3	1.2	0.190	0.057	0.635
	Survey 8	142	0	0	-	-	-
	Survey 9	100	2	2.0	0.307	0.071	1.318

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.40 (08APR2025 23:40 GMT)

Table 9.57 Univariable logistic regression on survey associated with CIG38 haplotype's infection in GH Kintampo - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	46	0	0	-	-	-
	Survey 7	158	2	1.3	Reference	-	-
	Survey 8	256	1	0.4	0.306	0.028	3.401
	Survey 9	300	3	1.0	0.788	0.130	4.765

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.39 (08APR2025 23:40 GMT)

KE Kombewa

The crude OR to define the relationship between *P. falciparum* CIG38 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.33 (presented in Table 9.58), for unvaccinated participants in Table 14.8.1.35 (presented in Table 9.59), and for vaccinated participants in Table 14.8.1.34 (presented in Table 9.60).

The crude OR to define the relationship between *P. falciparum* CIG38 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.36 (fixed breakpoint of Survey 7) and in Table 14.8.1.37 (model-identified breakpoint).

A significant OR of *P. falciparum* CIG38 haplotype infection was observed at Survey 9 compared to Survey 3 in the overall population (Table 9.58), at Survey 8 compared to Survey 3 in the unvaccinated population (Table 9.59), and at Survey 9 compared to Survey 6 in the vaccinated population (Table 9.60).

Significant OR was observed using fixed breakpoint of Survey 7 on post-breakpoint group = 0.690 (CI: 0.553 - 0.861) (Table 14.8.1.36), while model-identified breakpoint was estimated at Survey 7 (Table 14.8.1.37).

Table 9.58 Univariable logistic regression on survey associated with CIG38 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	21	5.3	Reference	-	-
	Survey 4	398	31	7.8	1.524	0.860	2.702
	Survey 5	402	24	6.0	1.146	0.627	2.094
	Survey 6	399	24	6.0	1.155	0.632	2.111
	Survey 7	400	16	4.0	0.752	0.386	1.463

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
	Survey 8	402	14	3.5	0.651	0.326	1.299
	Survey 9	401	8	2.0	0.368	0.161	0.840

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.33 (08APR2025 23:40 GMT)

Table 9.59 Univariable logistic regression on survey associated with CIG38 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	21	5.3	Reference	-	-
	Survey 4	398	31	7.8	1.524	0.860	2.702
	Survey 5	402	24	6.0	1.146	0.627	2.094
	Survey 6	309	18	5.8	1.116	0.584	2.134
	Survey 7	266	14	5.3	1.003	0.501	2.009
	Survey 8	163	8	4.9	0.931	0.404	2.148
	Survey 9	96	1	1.0	0.190	0.025	1.430

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.35 (08APR2025 23:40 GMT)

Table 9.60 Univariable logistic regression on survey associated with CIG38 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	6	6.7	Reference	-	-
	Survey 7	134	2	1.5	0.212	0.042	1.076
	Survey 8	239	6	2.5	0.361	0.113	1.149
	Survey 9	305	7	2.3	0.329	0.108	1.005

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.34 (08APR2025 23:41 GMT)

9.4.3.5.2. Trends in CIG38 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S vaccination status

GH Kintampo

The adjusted OR of *P. falciparum* CIG38 haplotype infection in GH Kintampo using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.46 (presented in Table 9.61).

The adjusted OR of *P. falciparum* CIG38 haplotype infection in GH Kintampo using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.47 (fixed breakpoint of Survey 7) and in Table 14.8.1.48 (model-identified breakpoint).

A significant OR (adjusted) was estimated for Survey 8 compared with Survey 3, while a significant OR adjusted for multiple infection (3.45; CI: 1.93 - 6.15) was noted for prevalence of *P. falciparum* CIG38 haplotype infection in GH Kintampo (Table 9.61). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.829 and post-breakpoint OR = 0.846; Table 14.8.1.47), while model-identified breakpoint was estimated at Survey 9, with a significant change in OR (adjusted) observed on the pre-breakpoint group = 0.793 (CI: 0.653-0.963) (Table 9.61).

No significant change in OR (adjusted) estimate was observed fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.829 and post-breakpoint OR = 0.846; Table 14.8.1.47), while model-identified breakpoint was estimated at Survey 9 (Table 14.8.1.48).

Table 9.61 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG38 haplotype's infection in GH Kintampo - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	25	6.2	Reference	-	-
	Survey 4	398	18	4.5	0.668	0.337	1.324
	Survey 5	399	9	2.3	0.655	0.278	1.545
	Survey 6	402	12	3.0	0.615	0.282	1.342
	Survey 7	399	5	1.3	0.425	0.145	1.248
	Survey 8	398	1	0.3	0.083	0.009	0.801
	Survey 9	400	5	1.3	0.477	0.108	2.104
Gender	Female	1373	40	2.9	Reference	-	-
	Male	1424	35	2.5	0.663	0.396	1.108
Age group	0.5-1Y	1257	21	1.7	Reference	-	-
	2-4Y	1540	54	3.5	1.593	0.891	2.846

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
RTS,S/AS01 vaccination status	Unvaccinated	2037	69	3.4	0.416	0.102	1.705
	Vaccinated	760	6	0.8	Reference	-	-
Multiple infection	Children infected with >1 haplotype	267	57	21.3	3.442	1.927	6.150
	Children infected with 1 haplotype	243	18	7.4	Reference	-	-
	Children not infected	2287	0	0	-	-	-

CI = confidence interval; GH = Ghana; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.46 (08APR2025 23:39 GMT)

KE Kombewa

The adjusted OR of *P. falciparum* CIG38 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.43 (presented in Table 9.62).

The adjusted OR of *P. falciparum* CIG38 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.44 (fixed breakpoint of Survey 7) and in Table 14.8.1.45 (model-identified breakpoint).

No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3. A significant OR adjusted for multiple infection (3.33; CI: 2.17 - 5.11) was noted for prevalence of *P. falciparum* CIG38 haplotype infection in KE Kombewa (Table 9.62). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 1.070 and post-breakpoint OR = 0.796; Table 14.8.1.44) while the model-identified breakpoint was estimated at Survey 9 (Table 14.8.1.45).

Table 9.62 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG38 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	21	5.3	Reference	-	-
	Survey 4	398	31	7.8	1.597	0.855	2.983

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
	Survey 5	402	24	6.0	1.469	0.761	2.835
	Survey 6	399	24	6.0	1.357	0.697	2.642
	Survey 7	400	16	4.0	1.005	0.480	2.101
	Survey 8	402	14	3.5	1.155	0.498	2.678
	Survey 9	401	8	2.0	0.668	0.250	1.787
Gender	Female	1445	68	4.7	Reference	-	-
	Male	1357	70	5.2	1.031	0.705	1.507
Age group	0.5-1Y	1268	47	3.7	Reference	-	-
	2-4Y	1534	91	5.9	1.373	0.905	2.083
RTS,S/AS01 vaccination status	Unvaccinated	2034	117	5.8	0.817	0.415	1.608
	Vaccinated	768	21	2.7	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	106	24.3	3.332	2.172	5.111
	Children infected with 1 haplotype	375	32	8.5	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG38 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.43 (08APR2025 23:39 GMT)

9.4.3.6. Infection with *Plasmodium falciparum* CIG39 haplotype

9.4.3.6.1. Trends in CIG39 haplotype infection using univariable logistic regression

GH Kintampo

The crude OR to define the relationship between *P. falciparum* CIG39 haplotype infection and survey year in GH Kintampo using univariable logistic regression is provided for overall population in Table 14.8.1.54 (presented in Table 9.63), for unvaccinated participants in Table 14.8.1.56 (presented in Table 9.64), and for vaccinated participants in Table 14.8.1.55 (presented in Table 9.65).

The crude OR to define the relationship between *P. falciparum* CIG39 haplotype infection and survey year in GH Kintampo using univariable piecewise logistic regression is provided in Table 14.8.1.57 (fixed breakpoint of Survey 7) and is provided in Table 14.8.1.58 (model-identified breakpoint).

The overall population in GH Kintampo showed a significant OR of *P. falciparum* CIG39 haplotype infection at Surveys 5 through 9 (Table 9.63).

Similarly, the unvaccinated population in GH Kintampo showed a significant OR of *P. falciparum* CIG39 haplotype infection at Surveys 5 through 9 (Table 9.64). P. falciparum CIG39 haplotype was not detected in the vaccinated population during Surveys 7 and 8, while no significant OR was observed at Survey 9 (Table 9.65).

Significant OR was observed using fixed breakpoint of Survey 7 on pre-breakpoint group = 0.612 (CI: 0.486 - 0.769) and post-breakpoint group = 0.658 (CI: 0.436 - 0.994) (Table 14.8.1.57). Similarly, significant OR was observed using model-identified breakpoint, estimated at Survey 8, on pre-breakpoint group = 0.603 (CI: 0.510 - 0.712) (Table 14.8.1.58).

Table 9.63 Univariable logistic regression on survey associated with CIG39 haplotype's infection in GH Kintampo - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	29	7.2	Reference	-	-
	Survey 4	398	20	5.0	0.679	0.377	1.221
	Survey 5	399	9	2.3	0.296	0.138	0.634
	Survey 6	402	8	2.0	0.260	0.118	0.577
	Survey 7	399	6	1.5	0.196	0.080	0.477
	Survey 8	398	1	0.3	0.032	0.004	0.238
	Survey 9	400	3	0.8	0.097	0.029	0.321

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.54 (08APR2025 23:40 GMT)

Table 9.64 Univariable logistic regression on survey associated with CIG39 haplotype's infection in GH Kintampo - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	401	29	7.2	Reference	-	-
	Survey 4	398	20	5.0	0.679	0.377	1.221
	Survey 5	399	9	2.3	0.296	0.138	0.634
	Survey 6	356	7	2.0	0.257	0.111	0.595
	Survey 7	241	6	2.5	0.328	0.134	0.801
	Survey 8	142	1	0.7	0.091	0.012	0.674
	Survey 9	100	1	1.0	0.130	0.017	0.963

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100

95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.56 (08APR2025 23:40 GMT)

Table 9.65 Univariable logistic regression on survey associated with CIG39 haplotype's infection in GH Kintampo - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	46	1	2.2	Reference	-	-
	Survey 7	158	0	0	-	-	-
	Survey 8	256	0	0	-	-	-
	Survey 9	300	2	0.7	0.302	0.027	3.399

CI = confidence interval; GH = Ghana
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.55 (08APR2025 23:40 GMT)

KE Kombewa

The crude OR to define the relationship between *P. falciparum* CIG39 haplotype infection and survey year in KE Kombewa using univariable logistic regression is provided for overall population in Table 14.8.1.49 (presented in Table 9.66), for unvaccinated participants in Table 14.8.1.51 (presented in Table 9.67), and for vaccinated participants in Table 14.8.1.50 (presented in Table 9.68).

The crude OR to define the relationship between *P. falciparum* CIG39 haplotype infection and survey year in KE Kombewa using univariable piecewise logistic regression is provided in Table 14.8.1.52 (fixed breakpoint of Survey 7) and in Table 14.8.1.53 (model-identified breakpoint).

No significant OR was estimated on surveys in KE Kombewa in the overall, unvaccinated, or vaccinated populations (Table 9.66, Table 9.67, and Table 9.68, respectively). No significant OR was estimated using fixed breakpoint of Survey 7 (pre-breakpoint OR = 0.967 and post breakpoint OR = 0.963) in KE Kombewa, while model identified breakpoint was estimated at Survey 8 (Table 14.8.1.52 and Table 14.8.1.53).

Table 9.66 Univariable logistic regression on survey associated with CIG39 haplotype's infection in KE Kombewa - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	14	3.5	Reference	-	-
	Survey 4	398	20	5.0	1.459	0.726	2.930
	Survey 5	402	15	3.7	1.069	0.509	2.244
	Survey 6	399	14	3.5	1.003	0.472	2.131

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
	Survey 7	400	14	3.5	1.000	0.470	2.126
	Survey 8	402	15	3.7	1.069	0.509	2.244
	Survey 9	401	13	3.2	0.924	0.429	1.991

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.49 (08APR2025 23:40 GMT)

Table 9.67 Univariable logistic regression on survey associated with CIG39 haplotype's infection in KE Kombewa - Unvaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	14	3.5	Reference	-	-
	Survey 4	398	20	5.0	1.459	0.726	2.930
	Survey 5	402	15	3.7	1.069	0.509	2.244
	Survey 6	309	11	3.6	1.018	0.455	2.274
	Survey 7	266	8	3.0	0.855	0.354	2.067
	Survey 8	163	7	4.3	1.237	0.490	3.124
	Survey 9	96	6	6.3	1.838	0.687	4.915

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.51 (08APR2025 23:41 GMT)

Table 9.68 Univariable logistic regression on survey associated with CIG39 haplotype's infection in KE Kombewa - Vaccinated participants - Analysis Set

					Unadjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 6	90	3	3.3	Reference	-	-
	Survey 7	134	6	4.5	1.359	0.331	5.581
	Survey 8	239	8	3.3	1.004	0.260	3.873
	Survey 9	305	7	2.3	0.681	0.173	2.690

CI = confidence interval; KE = Kenya
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N × 100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
Source: Table 14.8.1.50 (08APR2025 23:41 GMT)

9.4.3.6.2. Trends in CIG39 haplotype infection using multivariable logistic regression by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status

GH Kintampo

The adjusted OR of *P. falciparum* CIG39 haplotype infection in GH Kintampo using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S vaccination status) is provided in Table 14.8.1.62 (presented in Table 9.69).

The adjusted OR of *P. falciparum* CIG39 haplotype infection in GH Kintampo using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.63 (fixed breakpoint of Survey 7) and in Table 14.8.1.64 (model-identified breakpoint).

A significant OR (adjusted) was observed at Survey 6 compared with Survey 3, while a significant OR adjusted for multiple infection (2.50; CI: 1.45 - 4.30) was noted for prevalence of *P. falciparum* CIG39 haplotype infection in GH Kintampo (Table 9.69). A significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 on pre-breakpoint group = 0.728 (CI: 0.569 - 0.932) (Table 14.8.1.63) while model-identified breakpoint was estimated at Survey 9, with a significant change in OR (adjusted) estimate on the pre-breakpoint group = 0.762 (CI: 0.628 - 0.926) (Table 14.8.1.64).

Table 9.69 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01$_E$ vaccination status associated with CIG39 haplotype's infection in GH Kintampo - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
Survey	Survey 3	401	29	7.2	Reference	-	-
	Survey 4	398	20	5.0	0.629	0.330	1.200
	Survey 5	399	9	2.3	0.567	0.247	1.303
	Survey 6	402	8	2.0	0.349	0.149	0.817
	Survey 7	399	6	1.5	0.500	0.188	1.331
	Survey 8	398	1	0.3	0.141	0.016	1.255
	Survey 9	400	3	0.8	0.433	0.088	2.144
Gender	Female	1373	33	2.4	Reference	-	-
	Male	1424	43	3.0	1.067	0.642	1.773
Age group	0.5-1Y	1257	23	1.8	Reference	-	-
	2-4Y	1540	53	3.4	1.189	0.682	2.074
RTS,S/AS01 vaccination status	Unvaccinated	2037	73	3.6	1.238	0.244	6.277
	Vaccinated	760	3	0.4	Reference	-	-

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Multiple infection	Children infected with >1 haplotype	267	54	20.2	2.496	1.447	4.304
	Children infected with 1 haplotype	243	22	9.1	Reference	-	-
	Children not infected	2287	0	0	-	-	-

CI = confidence interval; GH = Ghana; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.62 (08APR2025 23:39 GMT)

KE Kombewa

The adjusted OR of *P. falciparum* CIG39 haplotype infection in KE Kombewa using multivariable logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.59 (presented in Table 9.70).

The adjusted OR of *P. falciparum* CIG39 haplotype infection in KE Kombewa using multivariable piecewise logistic regression (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) is provided in Table 14.8.1.60 (fixed breakpoint of Survey 7) and in Table 14.8.1.61 (model-identified breakpoint).

No significant OR (adjusted) was estimated in KE Kombewa for surveys compared with Survey 3, while a significant OR adjusted for multiple infection (2.59; CI: 1.63 - 4.09) was noted for prevalence of *P. falciparum* CIG39 haplotype infection in KE Kombewa (Table 9.70). No significant change in OR (adjusted) estimate was observed using fixed breakpoint of Survey 7 (pre-breakpoint OR = 1.013 and post-breakpoint OR = 1.134; Table 14.8.1.60) in KE Kombewa, while model-identified breakpoint was estimated at Survey 6) (Table 14.8.1.61).

Table 9.70 Multivariable logistic regression on survey adjusted on gender, age group, multiple infection and RTS,S/AS01E vaccination status associated with CIG39 haplotype's infection in KE Kombewa - Analysis Set

					Adjusted Odds Ratio		
						95% CI	
Characteristics	Categories	N	n	%	Value	LL	UL
Survey	Survey 3	400	14	3.5	Reference	-	-
	Survey 4	398	20	5.0	1.481	0.711	3.087
	Survey 5	402	15	3.7	1.301	0.597	2.840
	Survey 6	399	14	3.5	1.095	0.492	2.439
	Survey 7	400	14	3.5	1.319	0.583	2.982
	Survey 8	402	15	3.7	1.795	0.740	4.349

					Adjusted Odds Ratio		
						95% CI	
Characteristics	**Categories**	**N**	**n**	**%**	**Value**	**LL**	**UL**
	Survey 9	401	13	3.2	1.674	0.654	4.286
Gender	Female	1445	50	3.5	Reference	-	-
	Male	1357	55	4.1	1.119	0.735	1.706
Age group	0.5-1Y	1268	37	2.9	Reference	-	-
	2-4Y	1534	68	4.4	1.353	0.852	2.151
RTS,S/AS01 vaccination status	Unvaccinated	2034	81	4.0	0.701	0.355	1.384
	Vaccinated	768	24	3.1	Reference	-	-
Multiple infection	Children infected with >1 haplotype	437	76	17.4	2.583	1.630	4.093
	Children infected with 1 haplotype	375	29	7.7	Reference	-	-
	Children not infected	1990	0	0	-	-	-

CI = confidence interval; KE = Kenya; RTS,S/AS01$_E$ = Particulate antigen, containing both RTS and HBs antigen (S) proteins / GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome.
N = number of enrolled participants
n = number of participants with CIG39 haplotype's infection
% = n/N x100
95% CI = 95% Wald confidence interval, LL = Lower Limit, UL = Upper Limit
Survey to be used as reference is the earliest survey where the haplotype is detected.
The category 'Children not infected' regroups children with no-infection on haplotype reported and missing.
Source: Table 14.8.1.59 (08APR2025 23:39 GMT)

9.5. Adverse events/adverse reactions

The EPI-MAL-010 study re-used samples collected during the EPI-MAL-005 study. There was no potential to collect serious and non-serious AEs, or incidents related to any GSK product during the conduct of this research, as the minimum criteria needed to report AEs, pregnancy exposures, and incidents were not present in the data source. Therefore, no new safety issues were able to be detected from adverse events/reactions as part of this study.

10. DISCUSSION

10.1. Key results

The samples collected from GH Kintampo showed a steeper decline in the overall number of haplotypes detected postvaccination compared with KE Kombewa (GH Kintampo: from 340 haplotypes during Survey 3 to 71 haplotypes during Survey 9; KE Kombewa: from 361 haplotypes during Survey 3 to 168 haplotypes during Survey 9). However, it is essential to consider the overall inconsistent rates of sequencing failure across surveys with highest rates of failures in surveys (Surveys 5, 8, and 9), and the gradual increase in the vaccination coverage starting from Survey 6.

Longitudinal Prevalence:

- 3D7 haplotype prevalence in GH Kintampo overall, was highest during Survey 3 (6.23%; CI: 4.07 – 9.07) and showed a gradual decline to Survey 9 (0.25%; CI: 0.01 – 1.38) and was not detected during Survey 8. Among female participants, 3D7 haplotype prevalence was highest during Survey 3 (6.95%; CI: 3.75 – 11.59), while among male participants, it was highest in Survey 4 (6.45%; CI: 3.57 – 10.59) and was not detected during Survey 8 and 9. Among 0.5 to 1 year old participants, 3D7 haplotype prevalence was highest during Surveys 3 and 4 (3.85%; CI: 1.56 - 7.76, each) and not detected during Surveys 7, 8, and 9, whereas among 2 to 4 years old participants, it was highest during Survey 3 (8.22%; CI: 4.94 - 12.68) and not detected during Survey 8. Among unvaccinated participants, 3D7 haplotype prevalence was highest during Survey 3 (6.23%; CI: 4.07 - 9.07) and not detected during Survey 8, while 3D7 haplotype was not detected among vaccinated participants during Surveys 6, 7, 8, and 9.
- 3D7 haplotype prevalence in KE Kombewa fluctuated across surveys overall, where the highest prevalence was observed during Survey 9 (1.75%; CI: 0.7 – 3.56) and was not detected during Survey 7 (0%; CI: 0 – 0.92). Among female participants in KE Kombewa, 3D7 haplotype prevalence was highest during Survey 3 (1.88%; CI: 0.51 - 4.74) and not detected during Survey 7, while among male participants it was highest in Survey 9 (1.98%; CI: 0.54 - 4.99) and not detected during Surveys 3, 4, and 7. Among 0.5 to 1 year old participants, 3D7 haplotype prevalence was highest in Survey 9 (2.76%; CI: 0.9 - 6.33) and was not detected in Surveys 6 and 7, while among 2 to 4 years old participants it was highest in Surveys 3 and 5 (1.37%; CI: 0.28 - 3.95, each) and not detected in Surveys 4 and 7. Among unvaccinated participants, 3D7 haplotype prevalence was highest during Survey 8 (1.84%; CI: 0.38 - 5.28) and not detected during Survey 7, while among vaccinated participants it was 1.97% (CI: 0.73 - 4.23) during Survey 9, but was not detected during Surveys 6, 7, and 8.
- Haplotype CIG32 showed the highest prevalence at GH Kintampo overall, during Survey 4 (8.29% [CI: 5.78 - 11.45]) and most prevalent across Survey 5 (2.76%; CI: 1.38 - 4.88), Survey 6 (5.72%; CI: 3.66 - 8.46), Survey 7 (2.26%; CI: 1.04 - 4.24), and 8 (3.02% CI: 1.57 - 5.21). CIG32 was the most prevalent haplotype across most surveys in female participants (all except Survey 3) as well as among male participants (all except Surveys 3, 5, and 9). Among 0.5 to 1 year old participants, CIG32 was the most prevalent haplotype across most surveys (Surveys 4, 6, 7, 8, and 9), while CIG39 was the most prevalent haplotype across Surveys 3, 5, and 9. Among 2 to 4 years old participants, CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8. CIG32 was the most prevalent haplotype across Surveys 4, 5, 6, and 8 among unvaccinated participants, whereas among the participants who received RTS,S/AS01$_E$ vaccination, CIG32 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG38 was most prevalent across Surveys 7 and 9.
- Haplotype CIG32 was the most prevalent haplotype overall, across Survey 3 (9.25%; CI: 6.6 - 12.52), Survey 4 (11.31%; CI: 8.37 - 14.84), Survey 6 (10.78%; CI: 7.91 - 14.24), and Survey 9 (3.74%; CI: 2.11 - 6.09) at KE Kombewa.

Additionally, haplotype CIG33 showed >5% prevalence in all surveys except Surveys 8 and 9 at KE Kombewa (with highest prevalence in Survey 5 [8.46%; CI: 5.93 -11.62], Survey 7 [6.00%; CI: 3.88 - 8.8], and Survey 9 [3.74%; CI: 2.11 - 6.09]). Among female participants in KE Kombewa, CIG32 was the most prevalent haplotype during Surveys 3, 4, 6, and 7, while CIG33 was the most prevalent during Surveys 5, 7, and 9. Among male participants in KE Kombewa, CIG32 was the most prevalent haplotype across Surveys 3, 4, 5, and 9, while CIG33 was the most prevalent haplotype across Surveys 6 and 7. Among 0.5 to 1 year old participants, CIG32 was the most prevalent during Surveys 3, 4, 5, 7, and 9, whereas among 2 to 4 years old participants, CIG32 was the most prevalent haplotype across Surveys 3, 4, and 6, while CIG33 was the most prevalent haplotype across Surveys 5, 7, and 9. Among unvaccinated participants, CIG32 was the most prevalent haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most prevalent during Surveys 5, 7, and 9, whereas among vaccinated participants, CIG33 was the most prevalent haplotype across Surveys 6, 7, and 8, while CIG32 was most prevalent during Surveys 7 and 9.

Sensitivity analysis for prevalence:

- 3D7 haplotype prevalence, under worst-case scenario, was highest during Survey 3 (14.46%; CI: 11.17 - 18.29) and lowest during Survey 8 (4.02%; CI: 2.32 - 6.45) at GH Kintampo, while its prevalence was highest in Survey 5 (12.19%; CI: 9.16 - 15.79) and lowest in Survey 7 (6.75%; CI: 4.5 - 9.67) at KE Kombewa.
- Under the “worst-case scenario” assumption for sensitivity analysis at each time point, CIG32 was the most prevalent haplotype in Survey 4 (15.83%; CI: 12.38 - 19.79), Survey 5 (12.28%; CI: 9.23 - 15.91), Survey 6 (8.46%; CI: 5.93 - 11.62), Survey 7 (7.02%; CI: 4.71 - 9.98), and Survey 8 (7.04%; CI: 4.73 - 10.01) in GH Kintampo, while in KE Kombewa, the most prevalent haplotypes were CIG32 (Survey 3 [18.25%; CI: 14.59 - 22.39], Survey 4 [19.85%; CI: 16.04 - 24.11], Survey 6 [19.30%; CI: 15.54 - 23.52], and Survey 9 [13.72%; CI: 10.5 - 17.48]) and CIG33 (Survey 5 [19.65%; CI: 15.88 - 23.88], Survey 7 [12.75%; CI: 9.64 - 16.42], and Survey 9 [13.72%; CI: 10.5 - 17.48]).
- 3D7 haplotype prevalence, using a threshold of 50 reads, was highest during Survey 3 (5.99%; CI: 3.87 - 8.77) and was not detected during Survey 8 at GH Kintampo; while 3D7 haplotype prevalence was highest in Survey 9 (1.50%; CI: 0.55 - 3.23) and was not detected during Survey 7 at KE Kombewa.
- Using a threshold of 50 reads, CIG32 haplotype showed highest prevalence across Survey 4 (7.79%; CI: 5.35 - 10.87), Survey 5 (2.76%; CI: 1.38 - 4.88), Survey 6 (5.47%; CI: 3.46 - 8.17), Survey 7 (2.26%; CI: 1.04 - 4.24), and Survey 8 (3.02%; CI: 1.57 - 5.21) in GH Kintampo as well as Survey 3 (8.75%; CI: 6.17 - 11.96), Survey 4 (9.80%; CI: 7.06 - 13.15), Survey 6 (10.53%; CI: 7.69 - 13.96), and Survey 9 (3.49%; CI: 1.92 - 5.79) in KE Kombewa.

Longitudinal frequency:

- Haplotype 3D7 showed highest frequency overall, during Survey 7 (9.41%; CI: 4.15 - 7.71) and was not detected during Survey 8 at GH Kintampo. Among female participants, 3D7 haplotype frequency was highest in Survey 3 (8.67%;

CI: 4.7 - 14.36) and was not detected during Survey 8, whereas among male participants, it was highest in Survey 7 (10.64%; CI: 3.55 - 23.1) and not detected during Surveys 8 and 9. Among 0.5 to 1 year old participants, 3D7 haplotype frequency was highest during Survey 3 (7.95%; CI: 3.26 - 15.7) and not detected during Surveys 7, 8, and 9, while among 2 to 4 years old participants, it was highest during Survey 7 (11.94%; CI: 5.3 - 22.18) and not detected during Survey 8. Among unvaccinated participants, 3D7 haplotype frequency was highest during Survey 7 (10.67%; CI: 4.72 - 19.94) and not detected during Survey 8, whereas it was not detected among vaccinated participants (Surveys 6, 7, 8, and 9).

- Overall, 3D7 haplotype showed highest frequency during Survey 9 (4.17%; CI: 1.69 - 8.4) and was not detected during Survey 7 at KE Kombewa. Among female participants, 3D7 haplotype frequency was highest in Survey 9 (3.75%; CI: 0.78 – 10.57) and not detected during Survey 7, whereas among male participants it was highest in Survey 9 (4.55%; CI: 1.25 – 11.23) and not detected during Surveys 3, 4, and 7. Among 0.5 to 1 year old participants, the 3D7 haplotype frequency was highest in Survey 9 (7.58%; CI: 2.51 - 16.8) and not detected in Surveys 6 and 7, while among 2 to 4 years old participants, it was highest in Survey 9 (1.96%; CI: 0.24 – 6.9) and not detected in Surveys 4 and 7. Among unvaccinated participants, 3D7 haplotype frequency was highest during Survey 8 (3.16%; CI: 0.66 – 8.95) and not detected during Survey 7, while among vaccinated participants it was 6.19% (CI: 2.3 – 12.98) during Survey 9 and not detected during Surveys 6, 7, and 8.
- Haplotype CIG32 was the most frequently detected haplotype in GH Kintampo across most surveys, overall (Survey 4 [11.22%; CI: 7.85 - 15.4], Survey 5 [9.09%; CI: 4.63 - 15.68], Survey 6 [10.50%; CI: 6.77 - 15.34], Survey 7 [10.59%; CI: 4.96 - 19.15], and Survey 8 [18.75%; CI: 10.08 – 30.46]). CIG32 was the most frequently detected haplotype across most surveys among female participants (Surveys 4, 5, 6, 7, 8, and 9) as well as among male participants (Surveys 4, 6, 7, and 8). CIG32 was also the most frequently detected haplotype among 0.5 to 1 year old participants (all surveys except for Surveys 3 and 5) as well as among 2 to 4 years old participants (Surveys 4, 5, 6, and 8). Considering vaccination status, CIG32 was the most frequently detected haplotype across most surveys among unvaccinated participants (Surveys 4, 5, 6, and 8), whereas among vaccinated participants, CIG32 was most frequently detected across Surveys 7 and 8, while CIG38 was most frequently detected across Surveys 7 and 9.
- Haplotype CIG32 was the most frequently detected haplotype across most surveys overall, in KE Kombewa (Survey 3 [10.25%; CI: 7.32 - 13.85], Survey 4 [12.36%; CI: 9.16 - 16.19], Survey 6 [12.01%; CI: 8.83 - 15.84], and Survey 9 [8.93%; CI: 5.08 - 14.3]). CIG32 was the most frequently detected haplotype across most surveys among female participants (Surveys 3, 4, 6, and 7) as well as male participants (Surveys 3, 4, 5, and 9). Among 0.5 to 1 year old participants, CIG32 was the most frequent during most surveys (Surveys 3, 4, 5, 7, and 9). However, among 2 to 4 years old participants, CIG32 was the most frequent haplotype across Surveys 3, 4, and 6, while CIG33 was the most frequent haplotype across Surveys 5, 7, 8, and 9. Among unvaccinated participants, CIG32 was the most frequently detected haplotype across Surveys 3, 4, 6, and 8, while CIG33 was most frequently

detected during Surveys 5, 7, and 9. Among vaccinated participants, CIG33 was the most frequently detected haplotype across Surveys 6, 7, and 8.

Sensitivity analysis for frequency:

- 3D7 haplotype frequency using a threshold of 50 reads was highest during Survey 7 (8.64%) and was not detected during Survey 8 at GH Kintampo; while 3D7 haplotype frequency was highest in Survey 9 (3.77%) and was not detected during Survey 7 at KE Kombewa.
- Using a threshold of 50 reads, CIG32 haplotype had highest frequency across Survey 4 (11.40%; CI: 7.88 - 15.79), Survey 5 (10.68%; CI: 5.45 - 18.31), Survey 6 (10.09%; CI: 6.43 - 14.88), Survey 7 (11.11%; CI: 5.21 - 20.05), and Survey 8 (19.05%; CI: 10.25 - 30.91) at GH Kintampo, as well as in Survey 3 (10.39%; CI: 7.34 - 14.15), Survey 4 (11.27%; CI: 8.14 - 15.09), Survey 6 (12.14%; CI: 8.89 - 16.05), and Survey 9 (8.81%; CI: 4.9 - 14.33) at KE Kombewa.

Longitudinal prevalence trends for selected *P. falciparum* haplotypes:

- In the overall population in GH Kintampo, significant ORs of <1.0 for *P. falciparum* 3D7 haplotype were estimated on Surveys 5 to 9 (OR = 0.387 at Survey 5, OR = 0.384 at Survey 6, OR = 0.308 at Survey 7, and OR = 0.038 at Survey 9) compared to Survey 3, at statistical significance level of 0.05. A decrease in prevalence was observed from Survey 3 until Survey 9 with no case detected at Survey 8. No significant OR was estimated on surveys in the overall population in KE Kombewa.
- A significant OR of <1.0 for *P. falciparum* CIG32 haplotype prevalence was observed for the overall population during Surveys 7 and 9 (OR = 0.398 and OR = 0.130, respectively) compared to Survey 3, at statistical significance level of 0.05 in GH Kintampo. Significant ORs of *P. falciparum* CIG32 haplotype infection were detected at Surveys 7, 8 and 9 (OR = 0.571, OR = 0.250, OR = 0.381 respectively) compared to Survey 3 in the overall population in KE Kombewa.
- A significant OR of <1.0 for *P. falciparum* CIG33 haplotype prevalence was detected at Survey 5 through 9 (OR = 0.276, OR = 0.462, OR = 0.370, OR = 0.184, and OR = 0.183, respectively) compared to Survey 3 in the overall population in GH Kintampo. A significant OR for *P. falciparum* CIG33 haplotype infection was noted during Surveys 8 and 9 (OR = 0.380 and OR = 0.479, respectively) compared to Survey 3 in the overall population in KE Kombewa.
- CIG35 haplotype was not detected with ≥5% prevalence across all surveys at GH Kintampo, while no significant OR was estimated on surveys in the overall population, in KE Kombewa.
- A significant OR of <1.0 for *P. falciparum* CIG38 haplotype prevalence was observed for Surveys 5 through 9 (OR = 0.347, OR = 0.463, OR = 0.191, OR = 0.038, and OR = 0.190, respectively) compared to Survey 3, at statistical significance level of 0.05 for the overall population in GH Kintampo. A significant OR of *P. falciparum* CIG38 haplotype prevalence was observed at Survey 9 (OR = 0.368) compared to Survey 3 in the overall population in KE Kombewa.

- A significant OR of <1.0 for *P. falciparum* CIG39 haplotype prevalence was observed for Surveys 5 through 9 (OR = 0.296, OR = 0.260, OR = 0.196, OR = 0.032, and OR = 0.097, respectively) compared to Survey 3, at statistical significance level of 0.05 for the overall population and the unvaccinated population in GH Kintampo, but no significant OR was estimated on surveys in KE Kombewa.
- The analyses on pre-breakpoint group of surveys and on post-breakpoint group of surveys showed a linear decrease for 3D7 haplotype at GH Kintampo (OR on before and after breakpoint groups), observed for the unadjusted model. However, when the OR was adjusted for variables: gender, age group, RTS,S/AS01$_E$ vaccination status and multiple infection, the results were not significant. This could indicate that the observed decrease in ORs is potentially a correlation between variables and not only due to a decrease over time. For KE Kombewa, we observed that the post-breakpoint group was at Survey 7, which could be influenced by the higher prevalence of 3D7 haplotype in Survey 9 compared to prevalence of 3D7 haplotype in other surveys. However, the limitation here is that the model was forced to use this breakpoint as this was the survey when vaccination started. When there were several breakpoints, the first one given by the model would be the one around Survey 7).
- Multivariable piecewise logistic regressions (adjusted by gender, age group, multiple infection, and RTS,S/AS01$_E$ vaccination status) revealed for all the most frequent haplotypes that a multiple infection could be considered as a risk factor of infection for the most frequent haplotypes at KE Kombewa and GH Kintampo.

10.2. Limitations

The following limitations were described in the IR dated 26 November 2024, and are relevant for Surveys 8 and 9 as well.

Only samples that were identified as positive by malaria slide reading (performed on site) and/or by NAAT (performed at AMC in Amsterdam) were sent to HSPH/BI for sequencing. A proportion of *P. falciparum* positive samples did not produce sequencing results for potential different reasons:

- The possibility of sequencing false positive samples. Assuming the correlation between malaria slide reading and the NAAT technique was high but less than 100%, a limited number of samples being true negatives might not produce sequencing results.
- The potential sequence incompatibility between the primers that were used for polymerase chain reaction amplification/sequencing at HSPH/BI relative to some of the *P. falciparum* haplotypes infecting participants. Since *P. falciparum* genome is highly variable, there was a theoretical possibility that primers will not anneal efficiently on a subset of haplotype DNA leading to absence of sequencing results (false negative). Optimization of the technique was done at HSPH/BI to minimize this risk. Moreover, as both techniques have already been evaluated against local gold standards, this risk was considered negligible.
- Even though storage and sample shipping conditions were considered optimal in the framework of the EPI-MAL-005 [Neafsey, 2015] and the EPI-MAL-010 studies

(storage of filter paper samples at -20°C and shipped on dry ice and storage of extracted DNA at -80°C after NAAT), there was a theoretical possibility of DNA degradation through sample freezing/thawing. The impact of this possibility on the study results is assumed to be negligible, based on the observation of successful data generation rates in the MALARIA-066 study; as from 5,106 samples in the latter study, usable data was generated for 4,421 (86.6%) and 4,499 (88.1%) samples for the CSP and SERA2 amplicons, respectively. In this study (EPI-MAL-010), about 1322/1737 (approximately 76%) of collected samples in total (across surveys and the two sites) were evaluable.

RTS,S/AS01$_E$ vaccination being initially implemented in the framework of the MVIP, vaccine coverage and potential vaccine related pressure were expected to be limited at overall population level due to time-limited (about 18 months for the three-dose primary series) initial vaccination of children aged 5 to 12 months (at time of first dose) leading to:

- A gradual increase in the number of vaccinated children until the end of the MVIP resulting in an inconstant potential related vaccine pressure and short period of the study post vaccination.
- A limited proportion of the overall population being effectively vaccinated while *P. falciparum* infection affects all age groups.

The NAAT testing was conducted prior to sequencing (for the objectives of the ancestry study EPI-MAL-005), using the portion of the sample likely to contain the highest concentration of parasite DNA. So, the material available for sequencing may have been insufficient, particularly in true negative or low-parasitemia samples. This might have led to an underrepresentation of these cases in the sequencing dataset or bias the results.

The data collection and the estimation that has been produced may not be representative of the full genetic diversity across the year. The study results are based on data collected directly post-rain season. Adjustment to the difference in the length and the time of the rainy season for each site and within sites were not made. Also, the study assessed the possible impact of the vaccine on the genetic diversity of the parasite but did not include other antimalaria interventions in the assessment of the genetic diversity.

10.3. Interpretation of results

Overall, the results obtained in this study show that the proportion of participants who were eligible for sample sequencing (tested positive for *P. falciparum* by malaria blood slide reading and/or by NAAT) decreased at Survey 5, after implementation of the vaccination program, in Kintampo, Kenya, while the decrease was noted from Survey 7 and to a lesser extent in Kombewa, Ghana.

Burden of 3D7 Strain in Kombewa, Ghana versus Kintampo, Kenya:

Prevalence of *P. falciparum* 3D7 haplotype was higher in Kombewa Ghana than in Kintampo Kenya in the early surveys of the study. Ghana is a region known for 3D7 haplotype to be a predominant allele [Duah, 2016; Amegashie, 2020]. Ghana generally reported more cases of *P. falciparum* 3D7-type haplotype compared to Kenya, in available genetic studies [Waitumbi, 2009; Amegashie, 2020].

As observed, a decline in the prevalence of 3D7 haplotype in Kombewa (Ghana) started prior to vaccination. This decline continued post vaccination, including no cases during Survey 8. The detection of vaccine-mediated shifts in allele frequencies may be more likely in settings where vaccine-like alleles are more abundant [Waitumbi, 2009]. A similar observation of the decline of prevalence of 3D7 across surveys was not seen in Kenya, however the prevalence of 3D7 pre-vaccine introduction was lower compared to Ghana. Studies have been conducted on the genetic diversity of *P. falciparum* in Kenya. While a study in 2005 in Kisumu population (Kenya) showed that CSP sequences with significant identity to the 3D7 vaccine allele were rare [Waitumbi, 2009], the haplotype network analysis of the Kenyan isolates (from different studies and regions) revealed 69 haplotypes with the 3D7 reference being found as the most prevalent haplotype (2012-2014) [Maina, 2024]. More specific data on the burden of 3D7 strain from Kenya would be needed for a conclusive comparison.

No cases of 3D7 haplotype were reported among vaccinated cohorts in both Kintampo KE (except in survey 9) and Kombewa, GH. This observation might indicate that the vaccine played a role via vaccine-driven selection pressure; as part of the CSP of the 3D7 strain is part of the vaccine composition. A previous study showed that RTS,S/AS01 vaccine efficacy is significantly higher against parasites matching the vaccine's 3D7 CSP allele, demonstrating allele-specific efficacy [Neafsey, 2015]. However, this observation should be interpreted cautiously due to low vaccination coverage at the early surveys in the post-vaccination period, which only increased gradually until Survey 9.

Vaccination coverage over surveys and number of cases per vaccination status:

The proportion of enrolled children (0.5 to 4 years of age) who received at least 1 dose of RTS,S/AS01$_E$ vaccine increased from Survey 6 to Survey 9 (11.4% to 75.0% and 22.6% to 76.1% in GH Kintampo and KE Kombewa, respectively). Evidence shows that completing the 4-dose schedule for RTS,S/AS01 vaccine is important for protective immunity [Zacharia, 2024].

Overall, fewer cases associated with a specific haplotype were observed among vaccinated children compared to unvaccinated children. (see Table 14.6.2.7, Table 14.7.2.7, Table 14.6.3.10, Table 14.7.3.10, Figure 14.6.2.8, Figure 14.7.2.8, Figure 14.6.3.11, Figure 14.6.3.12, Figure 14.7.3.11, and Figure 14.7.3.12).

Potential RTS,S/AS01$_E$ vaccine impact on non-3D7 haplotypes:

As the immune system becomes more effective at targeting 3D7-type parasites in vaccinated individuals [Neafsey, 2015], this might allow non-3D7 haplotypes to increase in frequency - a process known as immune escape or allele replacement (Gomes, 2016). However, there was no significant evidence from the trend analysis of this study suggesting that other haplotypes are replacing 3D7 haplotype after vaccine introduction.

In general, a reduction in the overall number of *P falciparum* haplotypes was observed in the post-vaccination period (71 haplotypes and 168 haplotypes in Survey 9) compared to the pre-vaccination (340 haplotypes and 361 haplotypes in Survey 3), in GH Kintampo and KE Kombewa, respectively. Also, an overall downward trend for longitudinal prevalence of *P. falciparum* 3D7 haplotype infection as well as other frequently detected haplotypes post vaccine introduction was observed for the overall population at individual sites (Figure 9.1, Figure 9.3, Figure 14.8.2.8, and Figure 14.8.2.11).

The overall decrease in the genetic variability of haplotypes post vaccination with RTS,S could be explained by the efficacy of the RTS,S/AS01 malaria vaccine against mismatched strains (33.4%) [Neafsey, 2015]. Also, findings of Waitumbi et al. [2009], support that RTS,S/AS01 vaccine elicit effects against CSP alleles with sequence mismatching to 3D7 CSP sequence. The structural similarities in the antigens of different haplotypes may induce some level of cross-protection. However, studies of RTS,S/AS01 vaccine cross-protection demonstrate mixed evidence on this regard [Laurens, 2020].

The interpretation of the absolute numbers of the detected haplotypes and the frequency estimates of the different haplotypes should be approached carefully, given the fluctuating rates of sequencing failure across surveys, ranging between approximately 17.7% to 34.7% across surveys and sites (Table 14.8.2.1) and particularly marked increase in rates in the most recent surveys. Such inconsistencies may bias the observed distribution of haplotype counts across surveys.

Failure in a proportion of sequenced samples, even when they are confirmed positive by other methods such as microscopy or NAAT was reported [Neafsey, 2015]. Challenges of deploying next generation sequencing for malaria molecular surveillance include low quantities of parasite DNA and high human DNA contamination, particularly at low parasitemia [Hamilton, 2023]. In addressing the non-evaluable (failed to be sequenced) samples during the prevalence analysis, 2 hypotheses were tested: one assuming non-evaluable samples are negative (main analysis), and the other assuming they were positive (sensitivity analysis), for the haplotype detected, to better approximate the range where the true value of the prevalence may fall in.

Results of the sensitivity analysis:

In the worst-case scenario analysis, the estimated prevalence and statistical outcomes remained comparable to the outcomes in the main analysis. This might indicate that the results are robust to assumptions about the haplotype status of non-sequenced samples or insufficient power due to small sample size or low haplotype frequency.

It's been observed that the most frequent and/or prevalent haplotypes remained consistent across the 2 read thresholds (15 and 50). It could also suggest the credibility of the results and that the selected read thresholds are biologically meaningful and not arbitrary.

10.4. Generalisability

EPI-MAL-010 was a longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study, where genotyping was conducted on samples of participants aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at 2 sites before and after the start of RTS,S/AS01$_E$ vaccination.

Bias was possible due to variety of factors (e.g., low number of samples with sequencing results, limited vaccine coverage, timing of sampling).

11. OTHER INFORMATION

Not applicable.

12. CONCLUSIONS

This study monitored the genetic diversity in CS sequences in the *P. falciparum* parasite population (measured through both haplotype frequency and prevalence) before and after vaccine implementation in children aged 6 months to <5 years in both GH Kintampo and KE Kombewa. The number of detected haplotypes varied between the sites. Across the surveys, it was observed that number of observed haplotypes was declining, however this observation is confounded by the high sequencing failure rate in more recent surveys (up to 30%). The observed prevalence of detected *P. falciparum* haplotypes varied by site over the surveys. The observed frequency of detected *P. falciparum* haplotypes also varied by site over the surveys. There were other haplotypes, like CIG32, CIG33, and CIG38, whose prevalence (and frequency) fluctuated across surveys, however no clear trend could be observed. In general, no haplotype showed clear dominance over time, rather varying prevalence over time and no clear pattern observed in unvaccinated versus vaccinated children. Overall, the prevalence and frequency of detected 3D7 haplotype was higher in GH Kintampo across surveys compared to KE Kombewa. In particular, 3D7 prevalence varied from around 6% in Survey 3 among unvaccinated in GH Kintampo down to 0.25% in Survey 9, and upward from 0% in Survey 7 to 1.8% in Survey 8 in the unvaccinated in KE Kombewa.

Given the study limitations that include the lack of adjustment to transmission period related factors including the length of rain season and other anti-malaria control interventions, caution is advised when generalizing the findings to other settings or populations.

13. REFERENCES

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14. TABLES AND FIGURES

ANNEX 1 LIST OF STAND-ALONE DOCUMENTS

No.	Document Reference No	Date	Title
1.	TMF-14010784	24 September 2021	Protocol Amendment Administrative Change 1
2	TMF-14048984	01 October 2021	Protocol Amendment Administrative Change 1 Sponsor Approval
3.	TMF-22073917	02 June 2025	Study Administrative Table
4.	TMF-17016970 (Version 4.0)	18 January2024	Original Statistical Analysis Plan
5	TMF-17016970 (Version 5.0)	10 December 2024	Statistical Analysis Plan Amendment 1
6.	TMF-19489436	26 November 2024	Interim Report
7.	TMF-21732855	06 May 2025	Erratum to Interim Report
8.	RPS-LIT-005691	24 June 2025	Neafsey DE, Juraska M, Bedford T, et al. Genetic Diversity and Protective Efficacy of the RTS,S/AS01 Malaria Vaccine. N Engl J Med. 2015;373(21):2025-2037

List of other documents:

- Signature of co-sponsor approval
- Post-text tables from Interim Analysis report

ANNEX 2 ADDITIONAL INFORMATION

Not applicable.

Protocol Administrative Change 1 Final

Study ID: 205071

Official Title of Study: A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the Plasmodium falciparum parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.

Date of Document: 09-Jun-2020

PASS INFORMATION

Title:	A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
Protocol version identifier:	205071 (EPI-MALARIA-010 VS AME)
Date of last version of the protocol administrative change:	Administrative Change 1 Final: 24 September 2021
EU PAS Register No:	***EUPAS42948***
Active substance:	NA (Not applicable)
Medicinal product(s):	NA (Not applicable)
Product reference:	H0002300
Procedure number:	Not allocated
Opinion holder:	GlaxoSmithKline Biologicals Rue de l'Institut 89 1330 Rixensart, Belgium
Joint PASS	***No***
Research question and objectives:	**Co-primary objectives:** • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$. • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.

Countries of study:	The two pre-selected sites for the EPI-MALARIA-10 VS AME study (referred to as the EPI-MAL-010 study in the remainder of the protocol) are Kintampo in Ghana (Western Africa) and Kombewa in Kenya (Eastern Africa). The sites are located in the planned RTS,S/AS01$_E$ pilot implementation areas of Ghana and Kenya, which are moderate-to-high transmission areas as recommended by the World Health Organization (WHO) for the Malaria Vaccine Implementation Programme (MVIP).
Authors:	Coordinating author: • PPD (GSK Biologicals) GSK Biologicals contributing authors: • PPD, Epidemiology Lead (Aixial contractor for GSK Biologicals) • PPD, Senior Epidemiology Lead • PPD, Statistician (4clinics contractor for GSK) • PPD, Senior Epidemiology Expert • PPD, Epidemiology Lead (ManiAfriCare contractor for GSK) • PPD, Senior Manager, Clinical R&D • PPD, Lead Statistician • PPD, Study Delivery Lead • PPD, Oversight Data Manager • PPD, Clinical Safety Representative • PPD Clinical Laboratory Sciences (CLS) representative • PPD, Regulatory Affairs • PPD, Expert Scientist, Molecular Biology • PPD, Scientist, Molecular Biology

	Non GSK Biologicals contributing authors: • PPD, Harvard School of Public Health/ Broad Institute, United States • PPD, Academic Medical Centre (AMC), Amsterdam, The Netherlands

(Administrative Change 1, 24 September 2021)

OPINION HOLDER

Opinion holder:	GlaxoSmithKline Biologicals Rue de l'Institut 89, 1330 Rixensart, Belgium

(Administrative Change 1, 24 September 2021)

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2. LIST OF ABBREVIATIONS

AA	Amino Acid
AE	Adverse Event
AESI	Adverse Events of Special Interest
AMC	Academic Medical Centre (Amsterdam, The Netherlands)
AS01$_E$	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome
ATP	According-To-Protocol
BAM	Binary Alignment/Map
BI	Broad Institute
CI	Confidence Interval
CLS	Clinical Laboratory Sciences
CoI	Complexity of Infection
CS	Circumsporozoite
CSP	CircumSporozoite Protein
DNA	DeoxyriboNucleic Acid
EMA	European Medicines Agency
EoS	End of Study
EPI	Expanded Programme on Immunization
GPP	Good Pharmacoepidemiology Practice
GP	Genomics Platform
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface Antigen
HSPH	Harvard T. H. Chan School of Public Health
ICF	Informed Consent Form
ICH	International Conference on Harmonisation

IEC	Independent Ethics Committee
IRB	Institutional Review Board
LAR	Legally Acceptable Representative
LSLV	Last Subject Last Visit
MOH	Ministry Of Health
MPL	3'-O-desacyl-4'-monophosphoryl-lipid A (produced by GSK)
MTI	Malaria Transmission Intensity
MVIP	Malaria Vaccine Implementation Programme
NAAT	Nucleic Acid Amplification Test
NIH-NIAID	National Institute of Health - National Institute of Allergy and Infectious Diseases
OR	Odds Ratio
PASS	Post-Authorisation Safety Study
PATH-MVI	Program for Advanced Technologies in Health - Malaria Vaccine Initiative
PCD	Primary Completion Date
PCR	Polymerase Chain Reaction
P. falciparum	*Plasmodium falciparum*
QS-21	QS-21 Quillaja saponaria Molina, fraction 21
RNA	RiboNucleic Acid
RTS	Hybrid protein comprising HBs (hepatitis B surface antigen) and CSP portions
RTS,S	Particulate antigen, containing both RTS and HBs antigen (S) proteins
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SNP	Single Nucleotide Polymorphism

SSA	Sub-Saharan Africa
TFL	Tables, Figures, Listings
WHO	World Health Organization

3. RESPONSIBLE PARTIES

GSK Biologicals (GSK) has the overall responsibility for the conduct of the study.

PPD (Epidemiology Lead – Malaria) and PPD (Clinical and Epidemiology Program Lead – Malaria) are the GSK designated contact persons for this study.

4. ABSTRACT

Title	A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the Plasmodium falciparum parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
Version and date of the protocol administrative change	205071 (EPI-MALARIA-010 VS AME), Administrative Change 1 Final: 24 September 2021
Main authors	PPD (Epidemiology Lead – Malaria), PPD PPD (Senior Epidemiology Expert) and PPD (Senior Epidemiology Lead – Malaria) are the GSK Biologicals (GSK) designated contact persons for this study.
Rationale and background	GSK has developed a pre-erythrocytic *Plasmodium (P.) falciparum* malaria vaccine, RTS,S/AS01$_E$, for routine immunisation of infants and children living in malaria-endemic countries of Sub-Saharan Africa (SSA). RTS,S/AS01$_E$ will be the first vaccine to be implemented for the prevention of malaria and the first AS01-adjuvanted vaccine to be implemented in the paediatric population. The safety profile of RTS,S/AS01$_E$ has been evaluated during pre-authorisation clinical trials conducted mainly in Sub-Saharan Africa (SSA) and will be further monitored and evaluated during the Malaria Vaccine Implementation Programme (MVIP). GSK has developed a Post Approval Plan comprised of a set of complementary prospective studies (EPI-MALARIA-005 BOD AME [116682], referred to as the EPI-MAL-005 study in the remainder of the protocol, EPI-MALARIA-002 VS AME [115055], referred to as the EPI-MAL-002 study in the remainder of the protocol, and EPI-MALARIA-003 VS AME [115056], referred to as the EPI-MAL-003 study in the remainder of the protocol) that will be conducted in similar if not identical settings in order to expand the data on vaccine safety, effectiveness and impact of the RTS,S/AS01$_E$ vaccine. Study EPI-MAL-002, which started in Q4 2015, is a surveillance study before RTS,S/AS01$_E$ vaccination; study EPI-MAL-003 will monitor RTS,S/AS01$_E$ safety after vaccination, as well as vaccine effectiveness and impact, and

will only start when RTS,S/AS01$_E$ is registered, authorised in the country and implemented through an Expanded Programme on Immunization (EPI) schedule that builds on the routine national immunization programme, under the supervision of the Ministry of Health (MOH). Study EPI-MAL-005 is conducted to measure malaria transmission intensity (MTI) and the impact of vaccination on other malaria control interventions such as residual spraying and bed nets on a yearly basis during the rainy season. It started in Q4 2014, at the end of the rainy season in the West African sites with peak malaria transmission in the second half of the year, and in Q2 2015 in the East African sites with peak malaria transmission in the first half of the year. It will run until the completion of the EPI-MAL-003 study.

P. falciparum is a pathogen with high genetic variability and a high number of different circulating strains. The parasite has evolved mechanisms to vary cell surface antigens and elude the host's immune response. For this reason, there is a safety concern that RTS,S/AS01$_E$ vaccine selects specific parasite variants or alters the number of parasite haplotypes by exerting a selective pressure over time.

This ancillary study to EPI-MAL-005, referred to as the EPI-MAL-010 study, will monitor the genetic diversity in circumsporozoite sequences in the *P. falciparum* parasite population before and after vaccine implementation in children aged 6 months to <5 years.

Research question and objectives

Co-primary objectives

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.[1]
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.[2]

1 Haplotype prevalence is defined as the proportion of subjects infected with a specific haplotype detected by sequencing among both infected and non-infected subjects (i.e., the number of subjects with a specific haplotype divided by the total number of subjects included in EPI-MAL-010: both positive and negative subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study at the two sites.

2 Haplotype frequency is defined as the proportion of a specific haplotype as detected by sequencing among the individual malaria clones (i.e., number of occurrences with a specific haplotype divided by the total number of malaria clones).

Secondary objectives

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To estimate trends in longitudinal prevalence of *P. falciparum* in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To estimate trends in longitudinal frequency of *P. falciparum* in subjects aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.

Study design

- Type of design: Longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study. In order to characterise *P. falciparum* haplotypes, genotyping will be conducted on samples of subjects aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, one site in Eastern Africa and one site in Western Africa. Those sites will be the same during the entire duration of the study.
- Study population: Subjects aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at the two sites before and after the start of RTS,S/AS01$_E$ vaccination, may be included in the EPI-MAL-010 study.
- Biological samples: In the EPI-MAL-005 study, a blood sample is obtained for malaria blood slide reading conducted locally and 2 to 3 drops of blood are spotted onto filter paper for the Nucleic Acid Amplification Test (NAAT) (conducted at AMC [Academic Medical Centre], Amsterdam, The Netherlands). Both blood slide reading (by microscopy) and NAAT (from genomic deoxyribonucleic acid [DNA]) will be used to evaluate the level of asexual *P. falciparum* parasitaemia. The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study. For samples that are identified as positive for *P. falciparum* by malaria blood slide reading and/or by NAAT, a minimum of 15 microliters of DNA extracted at AMC will be sent to

Harvard T. H. Chan School of Public Health (HSPH)/ Broad Institute (BI) for amplicon sequencing.

- Sampling schedule: The EPI-MAL-010 study will re-use samples from subjects enrolled in 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study. Selection of surveys included in EPI-MAL-010 is contingent upon the start date of the MVIP and will require the first surveys to occur prior to MVIP start.
- Primary completion date (PCD): PCD is defined as the date of final collection of data for all primary outcomes.
- End of study (EoS): Last testing results released of samples re-used from EPI-MAL-005. EoS must be achieved no later than 8 months after the selection of the samples for testing of the last survey.
- Duration of the study: This design allows monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after the start of RTS,S/AS01$_E$ vaccination, across 7 consecutive annual surveys as described below:
  - 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

Note: Due to special circumstances (e.g. COVID-19 pandemic), some surveys might not be performed in some EPI-MAL-005 sites (samples not collected) meaning that not all sites in EPI-MAL-010 would have data from 7 consecutive annual cross-sectional surveys.

Population

The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study. Subjects aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, may be included in the EPI-MAL-010 study. The two pre-selected sites for the EPI-MAL-010 study are Kintampo in Ghana (Western Africa) and Kombewa in Kenya (Eastern Africa). These sites are located in the planned RTS,S/AS01$_E$ pilot implementation areas of Ghana and Kenya, which are moderate-to-high transmission areas as

recommended by the World Health Organization (WHO) for the MVIP.

Variables

Primary endpoint

- Occurrence of specific *P. falciparum* haplotype infection.
  - Criteria/definitions: infection with (a) particular *P. falciparum* haplotype(s) confirmed for the CS C-terminus and/or SERA2 loci using sequencing techniques.

Secondary endpoints

Secondary endpoints are identical to the primary endpoint. For trends analyses, only the 3D7 haplotype and the haplotypes with more than 5% of frequency will be considered.

Data sources

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study. In order to determine *P. falciparum* haplotypes, genotyping will be conducted on samples of subjects aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination.

Study size

The sample size is per design limited to the number of subjects included in the EPI-MAL-005 study. All eligible children below 5 years of age enrolled in EPI-MAL-005 during 7 cross-sectional surveys may be included in EPI-MAL-010 regardless of their parasitaemia status. Hence, 400 children below 5 years of age are expected to be enrolled every year in each of the two EPI-MAL-010 study sites.

Malaria prevalence was estimated at 25% for children aged 6 months to 5 years in the selected sites based on GSK sponsored studies in the same centres (data from the EPI-MAL-005 study had its first survey collected in 2014-2015).

Depending on the variations in parasite prevalence over the years, around 100 subjects are expected to have a positive malaria parasitaemia per site and per year.

Data analysis

- The haplotype prevalence will be estimated by site and per survey, as the number of subjects infected with a specific *P. falciparum* haplotype, divided by the total number of subjects. Thus, the denominator will be all the subjects aged 6 months to <5 years included in the EPI-MAL-010 study for each of the two sites considered: malaria positive AND negative subjects based on malaria blood reading and/or NAAT.

- The haplotype frequency will be estimated by site and per survey, as the number of occurrences of a specific *P. falciparum* haplotype, divided by the total number of clones. Thus, in case of multiple infections with *P. falciparum* malaria, the same subject will contribute multiple times in the denominator. The frequency will be estimated using data only from malaria positive subjects aged 6 months to <5 years included in the EPI-MAL-010 study for each of the two sites considered.

  All confidence intervals (CIs) will be two-sided 95% CIs computed using the exact method.

Milestones

The study is planned to be started in ***Q3 2021*** (start of sample selection), and to be ended in Q3 2024. The final report of study results is planned to be written by Q1 2025.
(Administrative Change 1, 24 September 2021)

5. AMENDMENTS AND UPDATES

Final: 9 June 2020

The rationale for the protocol administrative change 1 and the summary of changes are provided in Annex 4.

(Administrative Change 1, 24 September 2021)

6. MILESTONES

Milestone	Planned date
Start of sample selection	***Q3 2021***
End of data collection	Q3 2024
Interim report	Q1 2023*
Registration in the EU PAS register	***Q3 2021***
Final report of study results	Q1 2025

*Tentative if at least 4 different epochs after vaccination implementation are included in the study design.

(Administrative Change 1, 24 September 2021)

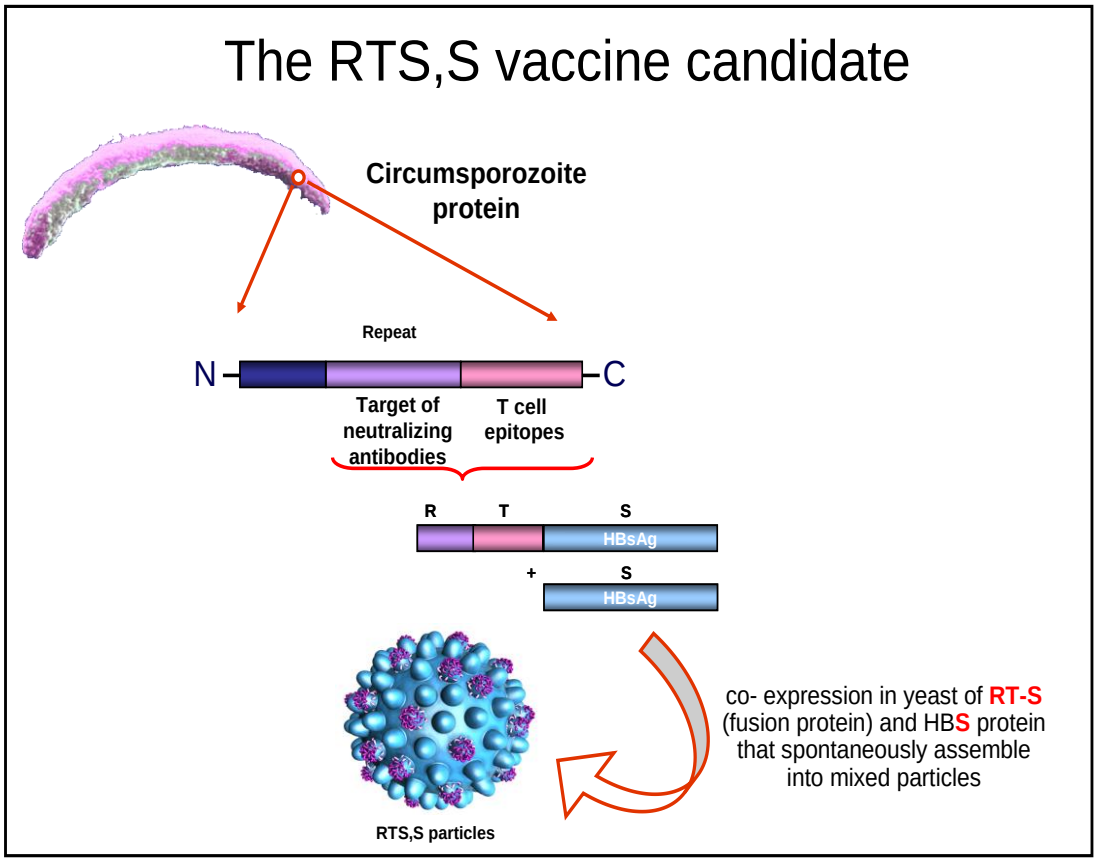
7. RATIONALE AND BACKGROUND

7.1. Background

GSK has developed a pre-erythrocytic *Plasmodium (P.) falciparum* malaria vaccine, RTS,S/AS01$_E$, for routine immunisation of infants and children living in malaria-endemic countries of Sub-Saharan Africa (SSA). RTS,S/AS01$_E$ will be the first vaccine to be implemented for the prevention of malaria and the first AS01-adjuvanted vaccine to be implemented in the paediatric population.

The vaccine antigen, RTS,S, is comprised of RTS (fusion protein containing the repeat portion and the C-terminus of the circumsporozoite [CS] protein of the clone 3D7, derived from the *P. falciparum* NF54 strain, and the amino terminal end of the hepatitis B surface antigen [HBsAg]) and of HBsAg co-expressed in *Saccharomyces cerevisiae* yeast expression system (Figure 1).

Figure 1 Schematic overview of the RTS,S antigen construct



The RTS,S/AS01$_E$ vaccine targets the 412 amino acids (AA) circumsporozoite protein (CSP) which is expressed on the *P. falciparum* sporozoite surface by early liver forms of the parasite cycle [Hoffman, 2015]. The vaccine contains the repetitive immunodominant B-cell epitope from the central region (NANP repeats) and the T-cell epitope containing C-terminal flanking region of CSP [Hoffman, 2015; Long, 2016]. The vaccine induces a specific antibody and CD4+ T-cell immune response against CSP which prevents blood-stage infection by neutralizing the parasites at the pre-erythrocyte stage [Hoffman, 2015; Long, 2016].

The antigen is adjuvanted with the AS01$_E$ Adjuvant System, which consists of two immune enhancers MPL (3'-O-desacyl-4'-monophosphoryl-lipid A) and QS-21 (Quillaja saponaria Molina, fraction 21), in a liposome suspension. The anti-CSP antibody response was significantly greater when the AS01$_E$ adjuvant was combined with the RTS,S as compared to the RTS,S in saline solution, due to the immune-enhancing properties of MPL and QS-21 [Leroux-Roels, 2014].

The pre-authorisation clinical development has been conducted mainly in SSA countries. The main clinical study supporting efficacy and safety is the large Phase III study, MALARIA-055 [110021], conducted at 11 study sites in 7 countries across SSA, which enrolled 15,459 children. In summary, a moderate protective efficacy against clinical disease and severe malaria that wanes over time was shown in the large Phase III study.

[The RTS,S Clinical Trials Partnership, 2011; The RTS,S Clinical Trials Partnership, 2012; The RTS,S Clinical Trials Partnership, 2014; The RTS,S Clinical Trials Partnership, 2015].

An ancillary genotyping study, MALARIA-066 [114831], was conducted as a supplement to the large Phase III study MALARIA-055. The aim of this ancillary study was to determine if the RTS,S/AS01$_E$ vaccine selects specific parasite variants. This post-vaccination study investigated the potential emergence or expansion of specific antigenic variants to enable vaccine evasion, by evaluating the genetic polymorphism of the CSP of *P. falciparum* found in infants and children who developed clinical malaria or with prevalent parasitaemia during one cross-sectional survey in different centres comparing vaccinees with controls. The study also measured the vaccine efficacy against the *P. falciparum* clone 3D7 haplotype compared with other *P. falciparum* haplotypes. There are different variants of the CSP in the parasite population. The central region of the CSP, containing NANP AA repeats that are also included in the RTS fusion-protein, is the main B-cell epitope of the CSP and is well conserved but variable in repeat count among different strains of *P. falciparum*. The C-terminus region, containing T-cell epitopes Th2R and Th3R that are also included in the RTS fusion-protein, is more polymorphic. The RTS protein contains the repeat portion and the C-terminus of the CSP of the clone 3D7. In MALARIA-066, these sections of the CSP from the *P. falciparum* parasites were sequenced in order to compare the haplotypes present in breakthrough infections to the haplotype of the vaccine strain.

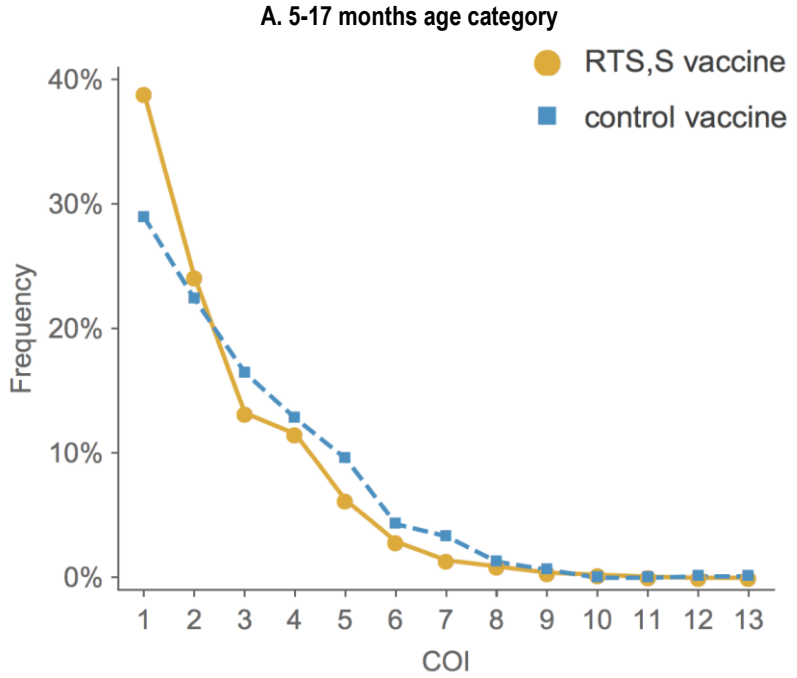
The MALARIA-066 study was conducted by the Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health (HSPH), and the Broad Institute (BI), in collaboration with GSK, and sponsored by the Program for Advanced Technologies in Health - Malaria Vaccine Initiative (PATH-MVI) and the National Institute of Health - National Institute of Allergy and Infectious Diseases (NIH-NIAID). The final results were reported in 2015 [Neafsey, 2015].

Three sets of results were generated: complexity of infection (CoI) – number of different haplotypes per infection, frequency of the 3D7 haplotype, and vaccine efficacy per portion of gene analysed. These three sets of results are briefly described below:

Complexity of infection

Sixty-eight percent of infants' (6-12 weeks age group) *P. falciparum* infections and 65% of children's (5-17 months age group) *P. falciparum* infections were caused by multiple infections (more than one haplotype infecting the same subject). In the 5-17 months age group, CoI was shifted toward fewer haplotypes in the vaccine group than in the control recipients (CoI ≥2: RTS,S/AS01$_E$ = 61% and control = 71%, Wald test P<0.001) (see Figure 2).

Figure 2 **Distribution of complexity of infections (CoI) in first or only clinical malaria cases (primary case definition, according-to-protocol [ATP] cohort)**



Data source: [Neafsey, 2015], Figure 3A.

Frequency of the 3D7 haplotype

The frequency of haplotypes with an exact match to the 3D7 vaccine strain varied between the sites. In East African sites, the 3D7 haplotype frequency was very low (≤ 7%) and in Western African sites, the frequency of 3D7 haplotype varied from 8 to 25%. These frequencies do not necessarily match the prevalence [Hastings, 2010; Malisa, 2012].

Vaccine efficacy

In the 5-17 months of age category, efficacy analyses over 12 months post Dose 3 suggested a slightly higher vaccine efficacy against 3D7 matched haplotypes compared to 3D7 mismatched haplotypes: vaccine efficacy, calculated using hazard ratios, against first or only episode of clinical malaria caused by *P. falciparum* with perfect C-terminus 3D7 match was 62.7% (95% confidence interval [CI]: 51.6 to 71.3) and with C-terminus 3D7 mismatch was 54.2% (95% CI: 49.9 to 58.1) (Wald sieve effect P = 0.06 for differential efficacy). It should be noted that the 95% CI of the efficacy for the matched cases is wide, due to the low frequency of the 3D7 vaccine haplotype and overlapping with the 95% CI of efficacy of the mismatched cases. Cumulative efficacy, calculated using proportion of subjects, gave lower efficacy values (50.3% and 33.4% for *P. falciparum* infection with or without perfect C-terminus 3D7 match, respectively) that reached a statistically significant difference (Wald sieve effect P = 0.04).

7.2. Rationale for the study

The safety profile of RTS,S/AS01$_E$ has been evaluated during pre-authorisation clinical trials conducted mainly in SSA and will be further monitored and evaluated during the Malaria Vaccine Implementation Programme (MVIP). GSK Biologicals (GSK) has developed a Post Approval Plan comprised of a set of complementary prospective studies (EPI-MAL-005 study, EPI-MAL-002 study and EPI-MAL-003 study) that will be conducted in similar if not identical settings in order to expand the data on vaccine safety, effectiveness and impact of the RTS,S/AS01$_E$ vaccine.

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 other adverse events (AEs) leading to hospitalisations and death. The first study, EPI-MAL-002, which started in Q4 2015, is a surveillance study before RTS,S/AS01$_E$ vaccination; the second study, EPI-MAL-003, which started in Q1 2019, monitors RTS,S/AS01$_E$ safety after vaccination, as well as vaccine effectiveness and impact, and started when RTS,S/AS01$_E$ was registered, authorised in the country and implemented through an Expanded Programme on Immunization (EPI) schedule that builds on the routine national immunization programme, under the supervision of the Ministry of Health (MOH). In parallel with both the EPI-MAL-002 and EPI-MAL-003 studies, a third study, EPI-MAL-005, is conducted to measure malaria transmission intensity (MTI) and the impact of vaccination on other malaria control interventions such as residual spraying and bed nets on a yearly basis during the rainy season. Study EPI-MAL-005 started in Q4 2014, at the end of the rainy season in the West African sites with peak malaria transmission in the second half of the year, and in Q2 2015 in the East African sites with peak malaria transmission in the first half of the year. It will run until the completion of the EPI-MAL-003 study.

P. falciparum is a pathogen with high genetic variability and a high number of different circulating strains. The parasite has evolved mechanisms to vary cell surface antigens and elude the host's immune response. For this reason, there is a safety concern that RTS,S/AS01$_E$ vaccine selects specific parasite variants or alters the number of parasite haplotypes by exerting a selective pressure over time.

This ancillary study to EPI-MAL-005, referred to as the EPI-MAL-010 study, will monitor the genetic diversity in circumsporozoite sequences in the *P. falciparum* parasite population before and after vaccine implementation in children aged 6 months to <5 years.

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Co-primary objectives

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.[3]
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.[4]

These co-primary objectives involve pharmacogenomics testing.

Refer to Annex 2 for the definition of pharmacogenomics.

Refer to Section 9.3.1 for the definition of the primary endpoint.

8.2. Secondary objectives

- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.
- To estimate trends in longitudinal prevalence of *P. falciparum* in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.
- To estimate trends in longitudinal frequency of *P. falciparum* in subjects aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.

These secondary objectives involve pharmacogenomics testing.

Refer to Annex 2 for the definition of pharmacogenomics.

Refer to Section 9.3.2 for the definition of the secondary endpoints.

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3 Haplotype prevalence is defined as the proportion of subjects infected with a specific haplotype detected by sequencing among both infected and non-infected subjects (i.e., the number of subjects with a specific haplotype divided by the total number of subjects included in EPI-MAL-010: both positive and negative subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study at the two sites.

4 Haplotype frequency is defined as the proportion of a specific haplotype as detected by sequencing among the individual malaria clones (i.e., number of occurrences with a specific haplotype divided by the total number of malaria clones).

9. RESEARCH METHODS

9.1. Study design

- Type of design: Longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MAL-005 study. In order to characterise *P. falciparum* haplotypes, genotyping will be conducted on samples of subjects aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, one site in Eastern Africa and one site in Western Africa. Those sites will be the same during the entire duration of the study.
- Study population: Subjects aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at the two sites before and after the start of RTS,S/AS01$_E$ vaccination, may be included in the EPI-MAL-010 study (refer to Section 9.2 for full details on study population and selection of samples).
- Biological samples: In the EPI-MAL-005 study, a blood sample is obtained for malaria blood slide reading conducted locally and 2 to 3 drops of blood are spotted onto filter paper for the Nucleic Acid Amplification Test (NAAT) (conducted at AMC [Academic Medical Centre], Amsterdam, The Netherlands). Both blood slide reading (by microscopy) and NAAT (from genomic deoxyribonucleic acid [DNA]) will be used to evaluate the level of asexual *P. falciparum* parasitaemia. The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study. For samples that are identified as positive for *P. falciparum* by malaria blood slide reading and/or by NAAT, a minimum of 15 microliters of DNA extracted at AMC will be sent to HSPH/BI for amplicon sequencing (see Section 9.2.8.2).
- Sampling schedule: The EPI-MAL-010 study will re-use samples from subjects enrolled in 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study. Selection of surveys included in EPI-MAL-010 is contingent upon the start date of the MVIP and will require the first surveys to occur prior to MVIP start.

Note: Due to special circumstances (e.g. COVID-19 pandemic), some surveys might not be performed in some EPI-MAL-005 sites (samples not collected) meaning that not all sites in EPI-MAL-010 would have data from 7 consecutive annual cross-sectional surveys.

- Primary completion date (PCD): PCD is defined as the date of final collection of data for all primary outcomes.

  Refer to Annex 2 for the definition of PCD.
- End of study (EoS): Last testing results released of samples re-used from EPI-MAL-005. EoS must be achieved no later than 8 months after the selection of the samples for testing of the last survey.

  Refer to Annex 2 for the definition of EoS.

- Duration of the study: This design allows monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after the start of RTS,S/AS01$_E$ vaccination, across 7 consecutive annual surveys as described below:
  - 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

Note: Due to special circumstances (e.g. COVID-19 pandemic), some surveys might not be performed in some EPI-MAL-005 sites (samples not collected) meaning that not all sites in EPI-MAL-010 would have data from 7 consecutive annual cross-sectional surveys.

Table 1 Study groups and epochs foreseen in the study

Study Group	Age (Min/Max)	Site (country)	Epochs, Surveys and predicted number of subjects*						
			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
Total	6 months to <5 years	Kintampo (Ghana)	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects
		Kombewa (Kenya)	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects	400 subjects

*The number of study subjects is approximate; of note, the number of subjects as presented in this table includes both *P. falciparum* malaria blood slide reading and/or NAAT positive and negative subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study in each of the two study sites. Sequencing techniques will only be applied on *P. falciparum* malaria blood slide reading and/or NAAT positive samples.

- Haplotype frequency is defined as the proportion of a specific haplotype as detected by sequencing among the individual malaria clones (i.e., number of occurrences with a specific haplotype divided by the total number of malaria clones).

  Haplotype prevalence is defined as the proportion of subjects infected with a specific haplotype as detected by sequencing among both infected and non-infected subjects (i.e., the number of subjects with a specific haplotype divided by the total number of subjects included in EPI-MAL-010: both positive and negative subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study at the two sites.

9.1.1. Discussion of study design

This design allows monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after MVIP start.

As explained above, genetic diversity of *P. falciparum* will be measured through both haplotype frequency and prevalence. The former estimates the annual distribution of a specific haplotype among all clones observed in the population, while the latter estimates the distribution of haplotypes among subjects aged 6 months to <5 years of age enrolled in the EPI-MAL-005 study at the two sites.

The number of vaccinated children among the study population is assumed to increase gradually after MVIP start. The RTS,S/AS01$_E$ vaccine is planned to be administered by the MOH through the EPI as a three-dose primary series, followed by a fourth booster dose. Based on this information and on the current assumption that the first three doses of RTS,S/AS01$_E$ should be administered to all subjects within a period of 18 months, conducting the EPI-MAL-010 study during 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study, pre- and post-RTS,S/AS01$_E$ MVIP start, should allow monitoring haplotype diversity in a representative proportion of subjects having received the vaccine. Vaccine coverage will be measured as described in the EPI-MAL-003 and EPI-MAL-005 study protocols.

Note: Due to special circumstances (e.g. COVID-19 pandemic), some surveys might not be performed in some EPI-MAL-005 sites (samples not collected) meaning that not all sites in EPI-MAL-010 would have data from 7 consecutive annual cross-sectional surveys.

9.2. Setting

9.2.1. Study population and number of subjects

Subjects aged 6 months to <5 years of age, enrolled in the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, may be included in the EPI-MAL-010 study.

The two pre-selected sites for the EPI-MAL-010 study are Kintampo in Ghana (Western Africa) and Kombewa in Kenya (Eastern Africa). The sites are located in the planned RTS,S/AS01$_E$ pilot implementation areas of Ghana and Kenya, which are moderate-to-high transmission areas as recommended by the WHO for the MVIP. Based on the results from MALARIA-066, selecting one site situated in Western Africa and the other in Eastern Africa will allow further exploring the already observed *P. falciparum* strain diversity differences between Western and Eastern Africa, as well as giving to EPI-MAL-010 a certain level of continental diversity in terms of human populations.

Each EPI-MAL-005 site aims to enrol about 600 children aged 6 months to 10 years per yearly cross-sectional survey, of which about 400 children will be aged 6 months to <5 years 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

Therefore, the total expected sample size per survey and per site is a maximum of 400 subjects aged 6 months to <5 years.

The number of subjects foreseen in the study is indicated in Table 1.

9.2.2. Samples selection

The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study. Only positive samples for *P. falciparum* infection will be sent to HSPH/BI for sequencing. Assuming a prevalence of *P. falciparum* infection of 25% in this age group (this prevalence figure has been evaluated by malaria slide reading after the EPI-MAL-005 study first cross-sectional survey), a total number of 100 positive samples per cross-sectional survey and per site are expected to be sent to HSPH/BI for sequencing.

The diagnostic testing for *P. falciparum* infection will be applied in parallel as shown in Figure 3 (see Annex 3), based on malaria blood slide reading and/or NAAT results as detected in the EPI-MAL-005 study.

Sequentially, the genotyping within the EPI-MAL-010 study will only be performed on *P. falciparum* positive (Pf+) samples (see Figure 3), whether *P. falciparum* positivity is obtained by malaria slide reading and/or NAAT.

9.2.3. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardise the scientific integrity and regulatory acceptability of the study 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 aged 6 months to <5 years enrolled in the EPI-MAL-005 study at two sites (one site in Eastern Africa and one site in Western Africa), fulfilling inclusion and exclusion criteria of the EPI-MAL-005 study.
- Subjects whose parent(s)/ Legally Acceptable Representative(s) [LAR(s)] have provided informed consent for the use of collected blood samples in further research as explained in the original informed consent form (ICF) of the EPI-MAL-005 study.

9.2.4. Exclusion criterion for enrolment

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

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

- All eligible subjects from the EPI-MAL-005 study that will be 5 years of age or over at time of the annual survey (i.e., at the time of sample collection) will be excluded.

9.2.5. Study period

In the framework of the MVIP, the RTS,S/AS01$_E$ vaccine is planned to be administered by the MOH through the EPI as a three-dose primary series, followed by a fourth booster dose. Based on this information and on the current assumption that the first three doses of RTS,S/AS01$_E$ should be administered to all subjects within a period of 18 months, conducting the EPI-MAL-010 study during 7 consecutive annual cross-sectional surveys of the EPI-MAL-005 study, pre- and post-RTS,S/AS01$_E$ MVIP start, should allow monitoring haplotype diversity in a representative proportion of subjects having received the vaccine.

Note: Due to special circumstances (e.g. COVID-19 pandemic), some surveys might not be performed in some EPI-MAL-005 sites (samples not collected) meaning that not all sites in EPI-MAL-010 would have data from 7 consecutive annual cross-sectional surveys.

9.2.6. Case definitions

In this study, a case is defined as a subject infected with any *P. falciparum* haplotype as detected by Illumina sequencing of polymerase chain reaction (PCR) amplicons.

- Infection with a haplotype is defined as the detection of more than 200 reads for the CSP C-terminus of *P. falciparum* for a single infection per sample. In a multiple infection sample, with at least 200 total reads for the CSP C-terminus amplicon, an infecting haplotype will be defined as a haplotype represented by at least 15 reads in samples with reads fewer than or equal to 1500 total, or 1% of all reads for a given sample with more than 1500 reads (observed at each amplicon following the data filtration of PCR and sequencing errors).
- Difference in haplotype: one single nucleotide polymorphism (SNP; ONLY non-synonymous encoding for a different AA) defines a different haplotype.

9.2.7. Study procedures

For each annual survey (Surveys 1 through 7), the following procedures will be performed for each subject:

- All applicable inclusion and exclusion criteria as described in Sections 9.2.3 and 9.2.4 will be checked.
- The ICF that was signed during the enrolment for the EPI-MAL-005 study will be checked to make sure that the parent(s)/LAR(s) gave their consent for further research on the study sample collected in the EPI-MAL-005 study (see also Section 10.1).
- Information regarding demographic data, vaccinations status, blood sampling, etc. will be obtained using an extraction from the EPI-MAL-005 database for all the subjects enrolled in EPI-MAL-010.
- For samples positive for *P. falciparum* infection (based on malaria blood slide reading and/or NAAT results as detected in the EPI-MAL-005 study), the extracted DNA prepared at AMC will be sent to HSPH/BI for sequencing (see also Section 9.2.2 and Annex 3).
- HSPH/BI will release sequencing results to GSK for statistical analysis.
- After sequencing HSPH/BI will destroy left over and/or non-used samples only after written approval by GSK.

The study will conclude when all the samples from subjects enrolled in Survey 7 at Year 7 have been sent to HSPH/BI and subjected to sequencing.

9.2.8. Biological sample handling and analysis

No samples will be collected specifically for this study. The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study.

Any sample testing will be done in line with the consent of the individual subject's parent(s)/LAR(s) in EPI-MAL-005.

Please refer to the EPI-MAL-005 and EPI-MAL-010 analytical plans for details of biospecimen management.

9.2.8.1. Biological samples

As described in Section 9.1, in the EPI-MAL-005 study a whole blood sample is obtained at the Survey Visits for malaria blood slide reading conducted locally and approximately 2 to 3 drops of blood are spotted onto filter paper for NAAT (conducted at AMC). Both blood slide reading (by microscopy) and NAAT (from genomic DNA) will be used to evaluate the level of asexual *P. falciparum* parasitaemia (refer to the biological samples Section 5.6.2 in the EPI-MAL-005 study protocol). For samples that are identified as positive for *P. falciparum* either by malaria blood slide reading and/or NAAT, a minimum of 15 microliters of DNA extracted at AMC will be sent to HSPH/BI for amplicon sequencing. Thus, there will be no sample collection from subjects during the EPI-MAL-010 study.

9.2.8.2. Laboratory assays

Please refer to Annex 3 for a detailed description of the assays performed in the study.

Other assays may be performed on extracted samples with the aim to investigate exploratory aspects of the study. The research may include, but is not limited to:

- Characterisation of the impact of vaccination on possible new parasitological markers;
- Translational research using next generation technologies.

These assays may explore the vaccine biological mechanism of action including vaccine-induced immunological response, and further understand the malaria disease using the next generation sequencing technologies.

Additional exploratory testing on the vaccine and/ or on the disease under study may be performed within the framework of the study if deemed necessary for accurate interpretation of the data, should such assay(s) become available at GSK. These assays may not be represented in the objectives/ endpoints of the study protocol.

9.3. Variables

9.3.1. Primary endpoint

- Occurrence of specific *P. falciparum* haplotype infection.
  - Criteria/definitions: infection with (a) particular *P. falciparum* haplotype(s) confirmed for the CS C-terminus and/or SERA2 loci using sequencing techniques.

9.3.2. Secondary endpoints

Secondary endpoints are identical to the primary endpoint. For trends analyses, only the 3D7 haplotype and the haplotypes with more than 5% of frequency will be considered (see Section 9.7.5 for details).

9.4. Data sources

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study.

In order to determine *P. falciparum* haplotypes, genotyping will be conducted on samples of subjects aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MAL-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, one site in Eastern Africa and one site in Western Africa.

Moreover, information regarding demographic data, vaccinations status, blood sampling, etc. will be obtained using an extraction from the EPI-MAL-005 database for all the subjects enrolled in EPI-MAL-010. Data collected in study EPI-MAL-005 are detailed in Section 5.5 of the EPI-MAL-005 study protocol.

9.5. Study size

9.5.1. Study size considerations

As presented in Section 9.2, the sample size is per design limited to the number of subjects included in the EPI-MAL-005 study. All eligible children below 5 years of age enrolled in EPI-MAL-005 during 7 cross-sectional surveys may be included in EPI-MAL-010 regardless of their parasitaemia status. Hence, 400 children below 5 years of age are expected to be enrolled every year in each of the two EPI-MAL-010 study sites.

Malaria prevalence was estimated at 25% for children aged 6 months to 5 years in the selected sites based on GSK sponsored studies in the same centres (data from the EPI-MAL-005 study had its first survey collected in 2014-2015).

Depending on the variations in parasite prevalence over the years, around 100 subjects are expected to have a positive malaria parasitaemia per site and per year.

9.5.2. Power considerations for prevalence analyses

Prevalence will be analysed at subject level, the sample size is defined as the total number of subjects per year and per study site. As described in Section 9.5.1, 400 subjects are expected to be included per site and per year.

The power for the prevalence analysis is computed using a sample size of 400 subjects per site and per year.

9.5.2.1. Prevalence estimates' precision

The expected precision around the estimation of the haplotype prevalence is presented in this section for a prevalence range of 0.5% to 10%. The range of 0.5% to 10% prevalence was chosen based on the prevalence of the 3D7 haplotype observed in the MALARIA-066 study and to cover scenarios involving more frequent haplotypes.

The expected range of the CI around different values of haplotype prevalence is computed in Table 2 considering a sample size of 400 children per site and per survey (all children with positive and negative parasitaemia are considered to compute the prevalence, see prevalence definition in Annex 2). CI limits are computed using the exact 95% CI.

Table 2 Exact 95% CI limits computed around different values of observed haplotype prevalence

Sample size	Exact	Expected haplotype prevalence			
	95% CI	0.5%	1%	5%	10%
400	LL (%)	0.1%	0.3%	3.1%	7.2%
	UL (%)	1.8%	2.5%	7.6%	13.4%

LL: Lower Limit; UL Upper Limit
Exact 95% CI limits computed with SAS 9.2.

9.5.2.2. Power considerations for prevalence trends analyses

Trends in longitudinal prevalence of *P. falciparum* haplotypes in subjects aged 6 months to <5 years, vaccinated or not with RTS,S/AS01$_E$, will be assessed by logistic model.

Power computations are based on the logistic model contrast analysing the variation in prevalence before and after vaccination implementation.

Power is computed based on a scenario with a total of 7 surveys, divided into 3 surveys before and 4 surveys after vaccination implementation.

The following parameters, mainly based on MALARIA-066 published and unpublished data, are also taken into account in the power computation:

- Proportions for prevalence trends analysis ranging from 0.5% to 10% (as described in Section 9.5.2.1).

- A 30% to 60% increase/decrease in prevalence before versus after vaccination implementation (on an indicative basis, the incidence of the 3D7 haplotype showed a 50% decrease after vaccination in MALARIA-066).

Power is computed for adjusted logistic regression models using the assumptions listed above, an alpha at 5% and an R-Square covariates parameter (R^2=0.2) to take into account the adjustment. Power computations for decreasing and increasing trends use similar assumptions.

Power computations are presented in Table 3 and Table 4 below, for decreasing trend and for increasing trend, respectively.

Table 3 Power to detect a range of decrease in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site

	Baseline haplotype prevalence			
Expected relative decrease*	**0.5%**	**1%**	**5%**	**10%**
30%	8%	12%	42%	72%
40%	12%	20%	68%	93%
50%	18%	29%	87%	99%
60%	25%	42%	97%	>99%

* Expected relative decrease between prevalence before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 87% to detect a relative prevalence decrease of 50% for haplotypes with a baseline prevalence of 5%, using a logistic regression analysis with an alpha of 5%.

Table 4 Power to detect a range of increase in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site

	Baseline haplotype prevalence			
Expected relative increase*	**0.5%**	**1%**	**5%**	**10%**
30%	6%	9%	32%	59%
40%	8%	13%	50%	82%
50%	10%	17%	67%	94%
60%	13%	22%	81%	99%

* Expected relative increase between prevalence before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 81% to detect a relative prevalence increase of 60% for haplotypes with a baseline prevalence of 5%, using a logistic regression analysis with an alpha of 5%.

9.5.3. Power considerations for frequency analyses

Frequency will be analysed at clone level, the sample size is defined as the total number of clones detected per year and per study site.

The number of detected clones expected per site and per year is based on the prevalence observed in the selected sites in the first year of EPI-MAL-005, and on the number of clones observed by subject in MALARIA-066 [Neafsey, 2015].

Assuming an average CoI of 3 clones per subject with positive parasitaemia, as computed based on MALARIA-066 data, and a malaria prevalence of 25% in both study sites as observed in EPI-MAL-005, 300 clones for 100 malaria positive subjects per site and per year are expected.

The power for frequency analysis is computed using a sample size of 300 clones per site and per year.

9.5.3.1. Frequency estimates' precision

The expected precision around the estimation of the haplotype frequency is presented in this section for a frequency range of 1% to 20%. Given the limited availability of frequency data for haplotypes other than 3D7, the range 1% to 20% was chosen to cover scenarios involving a range of rare and more frequent haplotypes.

The expected range of the CI around different values of haplotype frequency is computed in Table 5 considering a sample size of 300 clones, assuming an average CoI of 3 clones per subject as observed in the MALARIA-066 study, for 100 subjects with positive parasitaemia per site and per survey. CI limits are computed using the exact 95% CI.

Table 5 Exact 95% CI limits computed around different values of observed haplotype frequency

Sample size (clones)	Exact 95% CI	Expected haplotype frequency				
		1%	5%	10%	15%	20%
300	LL (%)	0.2%	2.8%	6.8%	11.2%	15.6%
	UL (%)	2.9%	8.1%	14.0%	19.6%	25.0%

LL: Lower Limit; UL Upper Limit
Exact 95% CI limits computed with SAS 9.2.

9.5.3.2. Power considerations for frequency trends analyses

Trends in longitudinal frequency of *P. falciparum* haplotypes in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$, will be assessed by logistic model.

Power considerations for frequency trends analyses are computed using the same method as for the prevalence trends analyses.

Power computations are based on the logistic model contrast analysing the variation in frequency before and after vaccination implementation.

Power is computed based on a scenario with a total of 7 surveys, divided into 3 surveys before and 4 surveys after vaccination implementation.

The following parameters, mainly based on MALARIA-066 published and unpublished data, are also taken into account in the power computation:

- Proportions for frequency trends analysis ranging from 1% to 20% (as described in Section 9.5.3.1).
- A 30% to 60% increase/decrease in frequency before versus after vaccination implementation (on an indicative basis, the incidence of the 3D7 haplotype showed a 50% decrease after vaccination in MALARIA-066).

Power is computed for adjusted logistic regression models using the assumptions listed above, an alpha at 5% and an R-Square covariates parameter (R^2=0.2) to take into account the adjustment. Power computations for decreasing and increasing trends use similar assumptions.

Power computations are presented in Table 6 and Table 7 below, for decreasing trend and for increasing trend, respectively.

Table 6 Power to detect a range of decrease in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 clones per survey and per site

	Baseline haplotype frequency			
Expected relative decrease*	**1%**	**5%**	**10%**	**20%**
30%	10%	34%	60%	90%
40%	16%	56%	85%	99%
50%	24%	77%	97%	>99%
60%	33%	92%	>99%	>99%

* Expected relative decrease between frequency before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 92% to detect a relative frequency decrease of 60% for haplotypes with a baseline frequency of 5%, using a logistic regression analysis with an alpha of 5%.

Table 7 Power to detect a range of increase in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 clones per survey and per site

	Baseline haplotype frequency			
Expected relative increase*	**1%**	**5%**	**10%**	**20%**
30%	8%	25%	47%	82%
40%	11%	39%	70%	97%
50%	14%	54%	86%	>99%
60%	18%	69%	95%	>99%

* Expected relative increase between frequency before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 86% to detect a relative frequency increase of 50% for haplotypes with a baseline frequency of 10%, using a logistic regression analysis with an alpha of 5%.

9.6. Data management

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study. The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study.

Data management in study EPI-MAL-005 is detailed in to Section 8.1 of the EPI-MAL-005 study protocol.

9.7. Data analysis

All the statistical calculations will be done in SAS 9.4 or higher.

Unless specified otherwise, all the statistical tests will be two-sided at alpha level of 0.05.

9.7.1. Datasets for analyses

9.7.1.1. Subject level total dataset

The subject level total dataset will include all subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study for the two considered sites, whose parent(s)/ LAR(s) have provided informed consent for the use of collected blood samples in further research.

9.7.1.2. Subject level according-to-protocol dataset

The subject level ATP dataset will include all evaluable (i.e., those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) malaria negative subjects and malaria positive subjects based on malaria blood reading and/or NAAT.

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

All prevalence analyses will be conducted using the subject level ATP dataset, unless otherwise detailed.

9.7.1.3. Clone level dataset

The clone level dataset will include all evaluable clones detected among malaria positive subjects compliant with the ATP definition.

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

All frequency analyses will be conducted using the clone level dataset, unless otherwise detailed.

9.7.2. 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 <2 years, 2 to <5 years.

9.7.3. Analysis of demographics

The number of subjects enrolled in EPI-MAL-010 as well as the number excluded from the subject level ATP dataset (see Section 9.7.1.2 for details) will be presented. Demographic characteristics (age and gender) will be summarised using descriptive statistics.

9.7.4. Analysis of co-primary objectives

Unless specified otherwise, the prevalence analyses will be done using the subject level ATP dataset and the frequency analyses will be done using the clone level dataset (see Sections 9.7.1.2 and 9.7.1.3, respectively, for details).

9.7.4.1. Haplotype prevalence among children aged 6 months to <5 years

The haplotype prevalence will be estimated by site and per survey, as the number of subjects infected with a specific *P. falciparum* haplotype, divided by the total number of subjects. Thus, the denominator will be all the subjects aged 6 months to <5 years included in the EPI-MAL-010 study for each of the two sites considered: malaria positive AND negative subjects based on malaria blood reading and/or NAAT.

All CIs will be two-sided 95% CIs computed using the exact method [Clopper, 1934].

Note that the denominator was chosen to extrapolate the information on proportion fluctuations at a population level when the frequency analysis will be used to describe variations within the genetic pool of haplotypes.

A best and worst case allocation will be done using the subject level total dataset (see Section 9.7.1.1 for details) for non-evaluable samples with the sequencing method (subjects with non-evaluable *P. falciparum* haplotype). In such case, 2 sensitivity analyses will be conducted. The first sensitivity analysis (best case allocation) will be done considering all the non-evaluable samples as positive samples for the haplotype considered. The second sensitivity analysis (worst case allocation) will be done considering all the non-evaluable samples as negative samples for the haplotype considered.

9.7.4.2. Haplotype frequency among children aged 6 months to <5 years

The haplotype frequency will be estimated by site and per survey, as the number of occurrences of a specific *P. falciparum* haplotype, divided by the total number of clones. Thus, in case of multiple infections with *P. falciparum* malaria, the same subject will contribute multiple times in the denominator. The frequency will be estimated using data only from malaria positive subjects aged 6 months to <5 years included in the EPI-MAL-010 study for each of the two sites considered.

As for the haplotype prevalence, the CIs will be two-sided 95% CIs computed using the exact method [Clopper, 1934].

9.7.5. Analysis of secondary objectives

Unless specified otherwise, the prevalence analyses will be done using the subject level ATP dataset and the frequency analyses will be done using the clone level dataset (see Sections 9.7.1.2 and 9.7.1.3, respectively, for details).

The estimation of the prevalence and the frequency by subgroups will be done for all the haplotypes.

The trends analysis will be done only on the 3D7 haplotype and the haplotypes with more than 5% of frequency. The following paragraph describes the method used for the selection of the haplotypes.

Haplotypes selection method for the trends analysis

In addition to the 3D7 haplotype, the haplotypes above a pre-defined threshold will be selected.

Data from the MALARIA-066 study [Neafsey, 2015] on the frequency of haplotypes for the CSP C-terminus amplicon demonstrate that there are five ‘common’ haplotypes, i.e., with a frequency above 5%, across 11 sites in 7 countries. The gap between these five haplotypes and the lesser common haplotypes could define the frequency threshold. Moreover, selection of a threshold lower than 5% of frequency would have such an

impact on power that it would limit the interpretability of the frequency analysis for these haplotypes. Therefore, haplotypes with more than 5% of frequency will be analysed.

The proposed strategy will be applied using the first survey data. Since changes over time cannot be excluded, the strategy will be adapted if necessary. For example, a haplotype that was initially below the 5% threshold might become >5% in later surveys (or vice versa). Details of the strategy of selection of the haplotypes for the analyses will be detailed in the Statistical Analysis Plan (SAP).

9.7.5.1. Prevalence analysis

The prevalence analysis will be done using the subject level ATP dataset (see Section 9.7.1.2 for details).

9.7.5.1.1. Prevalence estimation by subgroups

The prevalence and 95% CI will be estimated by gender, age group (categories displayed in Section 9.7.2) and vaccination status using the same method described in Section 9.7.4.1.

9.7.5.1.2. Trends and annual fluctuations in haplotype prevalence among children aged 6 months to <5 years

Trends and annual fluctuations in haplotype prevalence will be analysed using logistic regression. This method will allow studying trends without making assumptions regarding annual fluctuations.

Logistic regression analyses per site will only be conducted for the haplotype 3D7 and the haplotypes with more than 5% of frequency (see Section 9.7.5). No adjustment for multiple testing will be employed.

Logistic models will be used to describe the nature of the relationship between the dependent variable (probability of having *P. falciparum* infection with a given haplotype) and the survey's year. Crude odds ratio (OR) and 95% CI will be estimated using a univariable logistic regression. Adjusted OR and 95% CI will be estimated using a multivariable logistic regression model adjusted on gender, age groups and vaccination status. All longitudinal estimations will be used in the models.

In addition, contrasts will be used to describe specific variations such as differences in prevalence before and after vaccination implementation or differences between two specific surveys. The list of contrasts will be described in the SAP.

9.7.5.2. Frequency analysis

The frequency analysis will be done using the clone level dataset (see Section 9.7.1.3 for details).

9.7.5.2.1. Frequency estimation by subgroups

The frequency and its 95% CI will be estimated by gender, age group and vaccination status using the same method described in Section 9.7.4.2.

9.7.5.2.2. Trends and annual fluctuations in haplotype frequency among children aged 6 months to <5 years

The trends and annual fluctuations in haplotype frequency will be analysed per site and only for the haplotype 3D7 and the haplotypes with more than 5% of frequency (see Section 9.7.5) using logistic regression following the method described in Section 9.7.5.1.2.

Moreover, multinomial logistic regression will be used to describe the annual fluctuations in haplotype frequency. The 3D7 haplotype will be treated as the reference group for the dependent variable. The OR and 95% CI will be estimated for each model. Specific two-by-two comparisons will be made using contrast to characterise the differences between two surveys. The two-by-two comparisons will be described in the SAP.

9.8. 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).

9.9. 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.

9.9.1. Sequence of analyses

An interim analysis will be performed, assuming that at time of the interim analysis, at least 4 different epochs after vaccination implementation are planned to be included in the study design. This interim analysis will be performed using as clean data as possible from the 3 surveys before vaccination implementation and the first 2 surveys after vaccination implementation. Of note, the feasibility of the interim analysis will be assessed by the study team based on the COVID-19 pandemic impact on the data availability.

Final analysis will be carried out on clean data collected up to the end of the study period.

All objectives will be assessed in both interim and final analyses.

All analyses will be detailed in the SAP and the Tables, Figures, Listings (TFL) before the database freeze.

A first report will be written at the time of the interim analysis and an integrated report will be written at the end of the study.

9.9.2. Statistical considerations for interim analyses

The interim analysis will only be descriptive. Therefore, no adjustment of type I error is needed.

For the sample size required for each epoch, please refer to Section 9.5.

9.10. Quality control

EPI-MAL-010 is an ancillary study to the EPI-MAL-005 study. The EPI-MAL-010 study will re-use DNA samples collected and processed during the EPI-MAL-005 study.

Monitoring by GSK in study EPI-MAL-005 is detailed in to Section 8.2 of the EPI-MAL-005 study protocol.

9.10.1. Quality assurance

To ensure compliance with Good Pharmacoepidemiology Practice (GPP) 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.

9.11. Limitations of the research methods

- Only samples that are identified as positive by malaria slide reading (performed on site) and/or by NAAT (performed at AMC in Amsterdam) will be sent to HSPH/BI for sequencing. Therefore, theoretically, all selected samples should produce sequencing results. However, there is a possibility that *P. falciparum* positive samples might not produce sequencing results for different reasons:
  - The first reason is the possibility of sequencing false positive samples. Assuming the correlation between malaria slide reading and the NAAT technique is high but less than 100%, a limited number of samples being true negatives may not produce sequencing results.
  - A second reason is related to the potential sequence incompatibility between the primers that are used for PCR amplification/sequencing at HSPH/BI relative to some of the *P. falciparum* haplotypes infecting study subjects. Since *P. falciparum* genome is highly variable, there is a theoretical possibility that primers will not anneal efficiently on a subset of haplotype DNA leading to absence of sequencing results. Optimization of the technique has been done at

HSPH/BI to minimize this risk. Moreover, as both techniques have already been evaluated against local gold standards, this risk is considered negligible.

– Finally, the quality of DNA that will be sent to HSPH/BI. Even though storage and sample shipping conditions are considered optimal in the framework of the EPI-MAL-005 and the EPI-MAL-010 studies (storage of filter paper samples at -20°C and shipped on dry ice and storage of extracted DNA at -80°C after NAAT), there is a theoretical possibility of DNA degradation through sample freezing/thawing. However, the impact on the study results is assumed to be negligible, based on the observation of successful data generation rates in the MALARIA-066 study: from 5,106 samples, usable data was generated for 4,421 and 4,499 samples for the CSP and SERA2 amplicons, respectively. There is no reason to expect the incidence of technical issues to be any different in EPI-MAL-010.

As a result, only the subjects for which sequencing results can be obtained will be considered for haplotype frequency estimation. For prevalence estimation, sensitivity analyses are planned to include samples without sequencing results (see Section 9.7.4.1).

- RTS,S/AS01$_E$ vaccination being initially implemented in the framework of the MVIP, vaccine coverage and potential related pressure are expected to be limited at overall population level due to time-limited (about 18 months for the three-dose primary series) initial vaccination of children aged 5 to 12 months (at time of 1st dose) leading to:

  1. A gradual increase of the number of vaccinated children until the end of the MVIP resulting in an inconstant potential related vaccine pressure.
  2. A limited proportion of the overall population being effectively vaccinated while *P. falciparum* infection affects all age groups.

- The power of the trends analysis could be considered suboptimal for low prevalence/frequency and/or for small variations. Satisfactory power is estimated to be reached through a combination of factors (i.e., prevalence ≥5% and increase/decrease of at least 50%, or frequency of at least 5% combined with an increase/decrease of at least 50%). It is important to note that the computations presented are proposed to illustrate the power that could be reached for the secondary objective, i.e., to estimate trends in prevalence and frequency, and that this is a non-interventional study with exploratory, hypothesis-generating objectives.

- Interactions between subject characteristics and vaccination status will not be studied due to the limited power (see Sections 9.7.5.1.2 and 9.7.5.2.2). The results of the adjusted analyses will be interpreted descriptively.

- No adjustment for multiple testing will be done for the trends analyses (see Section 9.7.5) because of the exploratory nature of the study objectives. The results of the trends analyses will be interpreted descriptively.

9.12. Potential extension

The WHO-led MVIP is considering the possibility to extend the vaccination implementation period beyond the period planned for this cohort. However, decision about vaccine implementation strategy is the remit of the National Health Authorities and there is currently no certainty regarding the continuation of vaccination in the MVIP area. If the decision is made to continue vaccination in the MVIP area, and continuous vaccine coverage is achieved, the duration of EPI-MAL-010 could be extended by two years, to mirror the timelines of the EPI-MAL-005 study. Of note, this extension will depend on feasibility of maintaining capacity for sequencing the parasite amplicons during the entire study period; this is currently being explored with the BI in Harvard which is in charge of the sequencing. Note that power computed considering the potential extension from 7 surveys to 9 surveys (corresponding to the study extension) does not substantially increase (see details below).

The following power considerations are computed considering a potential extension from 7 surveys to 9 surveys (corresponding to the study extension) divided into 3 surveys before and 6 surveys after vaccination implementation.

These power computations are based on the same assumptions stated in Section 9.5.2.2 for prevalence analyses and in Section 9.5.3.2 for frequency analyses.

Power computations for prevalence analyses are presented in Table 8 and Table 9 below, for decreasing trend and for increasing trend, respectively. Power computations for frequency analyses are presented in Table 10 and Table 11 below, for decreasing trend and for increasing trend, respectively.

The potential extension from 7 surveys to 9 surveys does not show substantial increase in power. For example, power increases from 81% with 7 surveys (Table 4) to 86% when using 9 surveys (Table 9), for power computations to detect a relative increase of 60% for haplotypes with a baseline prevalence of 5%, using a logistic regression analysis with an alpha of 5%.

Table 8 Power to detect a range of decrease in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site (considering potential extension to 9 surveys)

	Baseline haplotype prevalence			
Expected relative decrease*	**0.50%**	**1%**	**5%**	**10%**
30%	10%	15%	49%	79%
40%	15%	24%	75%	96%
50%	22%	36%	92%	>99%
60%	31%	50%	99%	>99%

* Expected relative decrease between prevalence before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

Table 9 Power to detect a range of increase in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site (considering potential extension to 9 surveys)

	Baseline haplotype prevalence			
Expected relative increase*	**0.50%**	**1%**	**5%**	**10%**
30%	6%	9%	35%	65%
40%	8%	13%	55%	87%
50%	10%	18%	73%	97%
60%	13%	24%	86%	>99%

* Expected relative increase between prevalence before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

Table 10 Power to detect a range of decrease in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 clones per survey and per site (considering potential extension to 9 surveys)

	Baseline haplotype frequency			
Expected relative decrease*	**1%**	**5%**	**10%**	**20%**
30%	12%	39%	67%	94%
40%	20%	63%	90%	>99%
50%	29%	84%	99%	>99%
60%	41%	95%	>99%	>99%

* Expected relative decrease between frequency before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

Table 11 Power to detect a range of increase in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 clones per survey and per site (considering potential extension to 9 surveys)

	Baseline haplotype frequency			
Expected relative increase*	**1%**	**5%**	**10%**	**20%**
30%	8%	27%	53%	88%
40%	11%	43%	76%	98%
50%	14%	60%	91%	>99%
60%	18%	75%	97%	>99%

* Expected relative increase between frequency before vs after vaccination implementation.
Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

9.13. Other aspects

Not applicable.

10. PROTECTION OF HUMAN SUBJECTS

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

The EPI-MAL-010 study will be conducted in accordance with all applicable regulatory requirements, including all applicable subject privacy requirements.

GSK will obtain favourable opinion/approval to conduct the study prior to study start 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.

The EPI-MAL-010 study will re-use samples collected during the EPI-MAL-005 study. (refer to the EPI-MAL-005 study protocol for details about regulatory and ethical considerations, including the informed consent process).

In the EPI-MAL-005 study, freely given and written or thumbprinted informed consent must be obtained from each subject's parent(s)/LAR(s) or the impartial witness as appropriate, prior to participation in the study. For subjects whose parent(s)/LAR(s) agree that their child's/ward's samples may be used for future research, parasite sequencing techniques may be performed in the EPI-MAL-010 study using blood samples collected in the EPI-MAL-005 study. There will be no additional informed consent for the EPI-MAL-010 study.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

The EPI-MAL-010 study will re-use samples collected during the EPI-MAL-005 study. Therefore, no safety issues could occur as a part of this study. Regarding adverse events (AEs) or serious adverse events (SAEs) that could occur during the course of the EPI-MAL-005 study, please refer to the safety Section 6 in the EPI-MAL-005 study protocol.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A manuscript will be submitted to a peer reviewed journal for publication within the policy defined timelines.

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(Administrative Change 1, 24 September 2021)

Annex 1 List of stand-alone documents

No.	Document Reference No	Date	Title
1	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***	List of stand-alone documents
2	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***	Glossary of terms
3	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***	Laboratory assays
4	***205701 (EPI-MALARIA-010 VS AME)***	***24 September 2021***	***Administrative changes to the protocol***
5	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***	Protocol ***Administrative change*** Sponsor Signatory Approval
6.	***205071 (EPI-MALARIA-010 VS AME)***	***24 September 2021***	***ENCePP Checklist for study protocols***

(Administrative Change 1, 24 September 2021)

Annex 2 Glossary of terms

Adverse event:	Any untoward medical occurrence in a subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product, or temporally associated with a study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e., lack of efficacy), abuse or misuse.
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.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study **(Synonym of End of Trial)**	For studies without collection of Human Biologicals Samples or imaging data EoS is the Last Subject Last Visit (LSLV). For database studies, EoS is the date the database analysis is completed. For studies with collection/re-use of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after the start of the testing of the last survey
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.

eTrack:	GSK Biologicals' tracking tool for clinical/ epidemiological trials.
Haplotype:	A haplotype is defined as a specific constant sequence of amino acids (AAs). Two haplotypes or variants differ by a single nucleotide polymorphism (SNP) at loci that generate at least one AA mutation in the DNA sequence (referred to as a "non-synonymous" mutation).
Haplotype frequency:	The proportion of a specific haplotype as detected by sequencing among the individual malaria clones (i.e., number of occurrences with a specific haplotype divided by the total number of malaria clones).
Haplotype prevalence:	The proportion of subjects infected with a specific haplotype detected by sequencing among both infected and non-infected subjects (i.e., the number of subjects with a specific haplotype divided by the total number of subjects included in EPI-MAL-010: both positive and negative subjects aged 6 months to <5 years enrolled in the EPI-MAL-005 study at the two sites.
Non-interventional (observational) Human Subject Research:	Studies where medicinal products, should they be administered, are prescribed in normal (routine) medical practice. No medical care or medical/scientific procedures as required in a research protocol are administered to participants except as part of routine medical care.
Nucleic Acid Amplification Test (NAAT):	Although not yet considered as the gold standard, NAAT-based 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 implementaion. This will also provide comparability of data collected in this study with the technique likely to be favoured in clinical trials in the future.

Pharmacogenomics:	The International Conference on Harmonization (ICH) E15 Guidance for Industry defines pharmacogenomics as Study of variation of DNA and RNA characteristics as related to drug or treatment response. Pharmacogenetics, which is a subset of pharmacogenomics, is "the study of variations in DNA sequence as related to drug response." Pharmacogenomic biomarkers include germline (host) DNA and RNA as well as somatic changes (e.g., mutations) that occur in cells or tissues. Pharmacogenomic biomarkers are not limited to human samples but include samples from viruses and infectious agents as well as animal samples. The term pharmacogenomic experiment includes both the generation of new genetic or genomic (DNA and/or RNA) data with subsequent analysis as well as the analysis of existing genetic or genomic data to understand drug or treatment response (pharmacokinetics, safety, efficacy or effectiveness, mode of action). Proteomic and metabolomic biomarker research are not pharmacogenomics.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the trial was concluded according to the pre-specified protocol or was terminated. For database studies, PCD is the date of final collection of data for all primary outcomes, whether the trial was concluded according to the pre-specified protocol or was terminated.
Post-Authorisation Safety Study:	A pharmaco-epidemiological study or a clinical trial carried out in accordance with the terms of the marketing authorisation, conducted with the aim of identifying or quantifying a safety hazard relating to an authorised medicinal product. This includes all GSK sponsored non-interventional studies and clinical trials conducted anywhere in the world that are in accordance with the terms of the European marketing authorisation and where the investigation of safety is the specific stated objective. Note: The phrase 'In accordance with the terms of the European marketing authorisation' means that the product is used according to the European label (e.g., within the recommended dose range, the approved formulation, indication etc.).

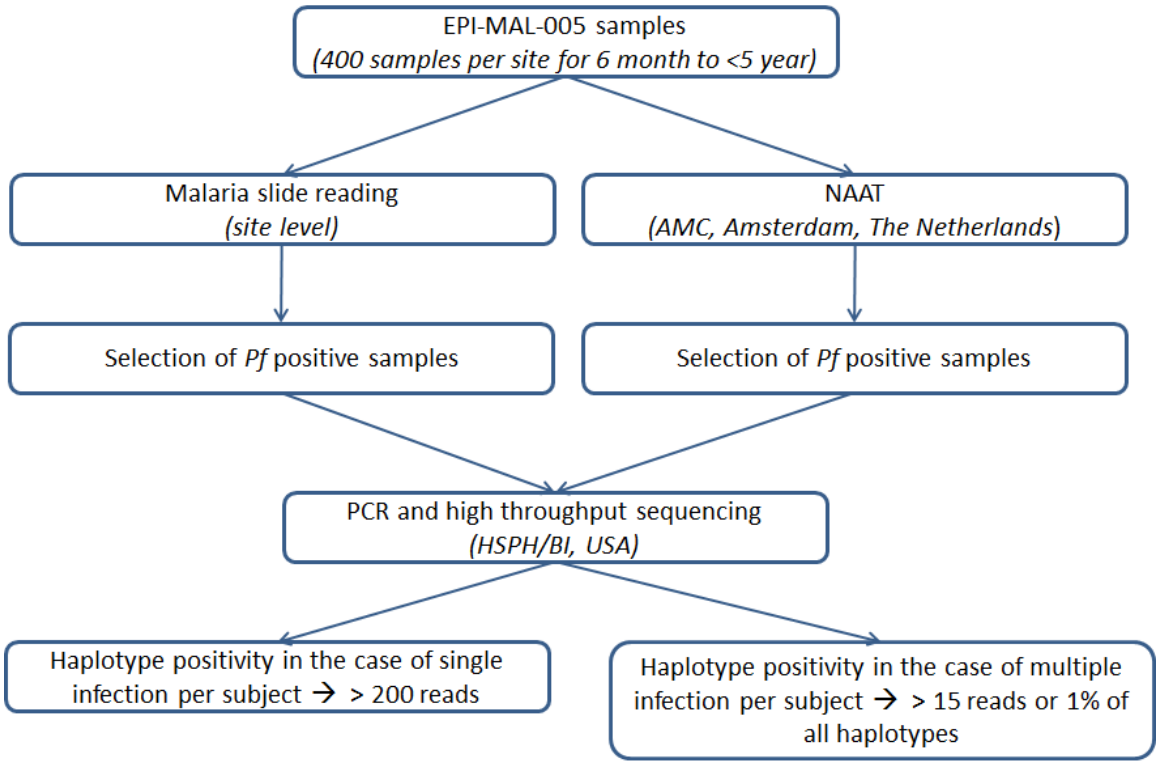
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or 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.
Research protocol:	A document that describes the objective(s), design, methodology, statistical considerations, and organisation of a study. The protocol usually also gives the background and rationale for the study, but these could be provided in other protocol referenced documents.
Retrospective study:	A study that looks backward in time (e.g., at events that occurred in the past; outcomes and exposure can no longer be influenced), usually using medical records, databases or interviews in order to address one or more study objectives.
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.
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.

Annex 3 Laboratory assays

Amplicon Sequencing Approach

For subjects identified as positive for malaria by blood slide reading and/or NAAT analysis, a minimum of 15 µl of DNA/sample extracted at AMC will then be sent to HSPH/BI in pre-barcoded sample tubes to be supplied by the BI. Custom workflows developed at the BI will be used to PCR amplify target regions of the DNA in each sample (described below), which will then be sequenced using the Illumina MiSeq platform. This process is referred to amplicon sequencing. The selection of samples and order of tests to be performed at the different locations is indicated in Figure 3.

Figure 3 Selection of samples and sequence of tests to be performed



NAAT = Nucleic Acid Amplification Test; AMC = Academic Medical Centre; *Pf* = *Plasmodium falciparum;* HSPH = Harvard T.H. Chan School of Public Health; BI = Broad Institute.

A high-throughput pipeline was developed at the BI to perform amplicon sequencing for the MALARIA-066 study from over 5,000 dried blood spot samples (see below, Amplicon Sequencing Methodology, and [Neafsey, 2015] for details on methodologies). This capacity yielded highly useful information about parasite samples that could not be obtained via any other practical means. By generating deep sequence coverage of hundreds to thousands of sequence reads from a collection of short (~300 nucleotides) PCR amplicons spanning highly variable antigenic regions from the *P. falciparum* genome, complete phasing information (i.e., ordering of the variable positions within each amplicon for each patient sample), was recovered. From this information, the haplotypes of common to extremely rare (~1% abundance) parasite strains within complex infections were reconstructed. Because the sequenced regions exhibit a high diversity of haplotypes at the parasite population level, it could be expected that genetically distinct parasites would only very rarely exhibit the same haplotype at more than one amplicon locus by chance, so the data therefore allowed the detection of all infections within patient samples with high sensitivity.

Parasite positivity is defined as samples returning > 200 sequencing reads mapping to at least one amplicon as performed at the HSPH/BI for single infections. In the case of multiple infections, the threshold for calling an individual haplotype within a parasite positive sample (which contains many haplotypes) is 15 reads (or 1% of all haplotypes from the same subject).

In our context, a haplotype is defined as a specific constant sequence of AAs. Two haplotypes or variants differ by SNP at loci that generate at least one AA mutation in the considered DNA sequence (non-synonymous mutations).

CSP C-terminus

In MALARIA-066, 26 major mutation points, with an allele frequency high enough for a powered allele-specific vaccine efficacy analysis, were identified in the CSP-terminus region among a sequence long of 111 AA. The DNA sequence is 333 base pairs long (including the primers) equivalent to 111 AA long that theoretically could generate several thousands of potential haplotypes.

SERA2:

The non-vaccine-target antigens such as SERA2 sequences will serve as a control for changes in CSP 3D7 haplotype frequency. If the parasite population changes in size due to a bottleneck during the course of the study, the frequency of all haplotypes will change stochastically, and this effect should manifest equally at CSP.

NANP/NVDP repeats:

NANP/NVDP repeats could define new haplotypes (by insertion/deletion). In MALARIA-066 it varied from 37 up to 43-44 repeats, but no association between repeat counts and vaccination efficacy was observed, and therefore, we do not plan to include this variable in the EPI-MAL-010 study analyses.

Amplicon Sequencing Methodology

Custom workflows developed at the BI will be used to PCR amplify CSP C-terminus and SERA2 target regions of DNA from each positive sample, and sequence these via the Illumina MiSeq platform.

A volume of 15 µl of DNA extracted at AMC from blood spotted onto Whatman Flinders Technology Associates (FTA) cards tagged with barcodes will be batched and shipped according to an agreed schedule from AMC to the BI Genomics Platform (GP) under a Center for Disease Control import permit. GP barcodes will be used to identify the samples, which will be registered in the GP laboratory information management system, and the samples will be stored securely at -20°C until further processing. The DNA concentration of each sample will be quantified using standard automated PicoGreen quantification. Samples will be batched into groups, typically 384 (4 sets of 96), with a negative and positive control sample. CSP C-terminus and SERA2 target regions will be PCR amplified from each sample. For each batch, PCR product pools will be normalized and combined into a single pool for Illumina sequencing. Sequencing data will be processed through the BI sequencing analysis pipeline generating standard sequencing metrics (e.g., read counts) and demultiplexed sample specific sequencing read Binary Alignment/Map (BAM) files. BAM files will be screened and filtered for human contaminating sequences, and packaged for submission to the National Center for Biotechnology Information short read archive database. All steps will be tracked in the laboratory information management system.

Annex 4 Administrative changes to the protocol

GlaxoSmithKline Biologicals **Vaccine Value & Health Science (VVHS)** Protocol Administrative Change 1	
eTrack study number and Abbreviated Title	205071 (EPI-MALARIA 010 VS AME)
Administrative Change number:	Administrative Change 1
Administrative Change date:	24 September 2021
Co-ordinating author:	PPD Lead Scientific Writer (GSK Biologicals)

Rationale/background for changes:
EPI-MALARIA 010 is referenced as a category 3 study in the 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 delete the introductory wording in Section 1 stating that the study was not a PASS and include the EU PAS registration information.

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

~~Protocol~~ *PASS* Information

~~This protocol is based on the European Medicines Agency (EMA) Guidance for the format and content of the protocol of non-interventional post-authorisation safety studies (PASS) [European Medicine Agency, 2016]. However, this study is not a PASS but will serve to evaluate the genetic diversity in the *Plasmodium falciparum* (*P. falciparum*) parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects.~~

EU PAS Register No:	***EUPAS42948*** ~~NA (Not applicable)~~
Joint PASS	***No***

OPINION HOLDER

Opinion holder:	GlaxoSmithKline Biologicals Rue de l'Institut 89, 1330 Rixensart, Belgium

2 Abstract:

Milestones	The study is planned to be started in ***Q3*** ~~2020~~ ***2021*** (start of sample selection), and to be ended in Q3 2024. The final report of study results is planned to be written by Q1 2025.

Section 5 Amendments and Updates

~~None.~~

Final: 9 June 2020

The raionale for the protocol administrative change 1 and the summary of changes are provided in Annex 4.

Section 6 Milestones

Milestone	Planned date
Start of sample selection	~~***Q4 Q3 20202021***~~
End of data collection	Q3 2024
Interim report	Q1 2023*
Registration in the EU PAS register	***Q3 2021*** ~~Not applicable~~
Final report of study results	Q1 2025

Section 13 References

~~European Medicines Agency. Guideline on good pharmacovigilance practices (GVP). Module VIII – Post-authorisation safety studies. 4 August 2016. EMA/813938/2011 Rev 2* Corr**.~~
~~http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/06/WC500129137.pdf. Accessed 20 July 2017.~~

Annex 1 List of stand-alone documents

No.	Document Reference No	Date	Title
1	205071 (EPI-MALARIA-010 VS AME)	~~09 June 2020~~***24 September 2021***	List of stand-alone documents
2	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***~~09 June 2020~~	Glossary of terms
3	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***~~09 June 2020~~	Laboratory assays
4	***205701 (EPI-MALARIA-010 VS AME)***	***24 September 2021***	***Administrative changes to the protocol***
5	205071 (EPI-MALARIA-010 VS AME)	***24 September 2021***~~09 June 2020~~	Protocol ***Administrative change*** Sponsor Signatory Approval

6.	***205071 (EPI-MALARIA-010 VS AME)***	***24 September 2021***	***ENCePP Checklist for study protocols***

Annex 4 Administrative changes to the protocol added.

Annex 6 ENCePP checklist for study protocols added

Annex 5 Protocol Administrative Change 1 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	205071 (EPI-MALARIA-010 VS AME)
Date of protocol	Administrative Change 1 Final: 24 September 2021
Detailed Title	A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
Sponsor signatory (GSK Biologicals)	François Roman, Clinical and Epidemiology Research & Development Project Lead
Signature	_____
Date	_____
Sponsor signatory (Harvard T. H. Chan School of Public Health/ Broad Institute)	Dyann Wirth, Professor
Signature	_____
Date	_____

Annex 6 ENCePP checklist for study protocols

Section 1: Milestones	***Yes***	***No***	***N/A***	***Page Number(s)***
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection	☒	☐	☐	***20***
1.1.2 End of data collection	☒	☐	☐	***20***
1.1.3 Study progress report(s)	☐	☒	☐	***20***
1.1.4 Interim progress report(s)	☒	☐	☐	
1.1.5 Registration in the EU PAS register	☒	☐	☐	***1***
1.1.6 Final report of study results.	☒	☐	☐	***20***

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)	☒	☐	☐	***20-24***
2.1.2 The objective(s) of the study?	☒	☐	☐	***25***
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	***29-31***
	☒	☐	☐	***41***
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:

Although it was requested to be added to the RMP to address a safety risk. This study is primarily an exploratory observational study addressing a safety risk

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)	☒	☐	☐	***26-29***
3.2 Does the protocol specify the primary and secondary (if applicable) endpoint(s) to be investigated?	☒	☐	☐	***34***
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)	☒	☐	☐	***40-43***

Comments:

Section 4: Source and study populations	***Yes***	***No***	***N/A***	***Page Number(s)***
4.1 Is the source population described?	☒	☐	☐	***29-30***
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period?	☒	☐	☐	***31***
4.2.2 Age and gender?	☒	☐	☐	***31***
4.2.3 Country of origin?	☒	☐	☐	***31***
4.2.4 Disease/indication?	☒	☐	☐	***31***
4.2.5 Co-morbidity?	☒	☐	☐	***31***

4.2.6 Seasonality?				
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	***31***

Comments:

The seasonality aspect of the study population recruitment and sampling is addressed in the EPI-MAL-005 protocol from whom this study is ancillary

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?	☒	☐	☐	***32***
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)	☒	☐	☐	***35-39***

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)	☐	☐	☒	
7.2 Does the protocol address known effect modifiers? (e.g. collection of data on known effect modifiers, anticipated direction of effect)	☐	☐	☒	

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.)	☒	☐	☐	***34***
8.1.2 Endpoints? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview	☒	☐	☐	***34***

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.)***	☒ ☒ ☒	☐ ☐ ☐	☐ ☐ ☐	***35-39***
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?	☒	☐	☐	***34-40***

Comments:

Section 10: Analysis plan	***Yes***	***No***	***N/A***	***Page Number(s)***
10.1 Does the plan include measurement of excess risks?	☒	☐	☐	***39-43***
10.2 Is the choice of statistical techniques described?	☒	☐	☐	***39-43***
10.3Are descriptive analyses included?	☒	☐	☐	***39-43***
10.4Are stratified analyses included?	☒	☐	☐	***39-43***
10.5 Does the plan describe methods for adjusting for confounding?	☒	☐	☐	***39-43***
10.6 Does the plan describe methods addressing effect modification?	☒	☐	☐	***39-43***

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?	☒	☐	☐	***39***
11.2 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	***39,44***
11.3 Are methods of quality assurance described?	☒	☐	☐	***44***
11.4 Does the protocol describe possible quality issues related to the data source(s)?	☒	☐	☐	***44***
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?	☒	☐	☐	***44-45***
12.1.2 Information biases?	☒	☐	☐	***44-45***
(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)	☒	☐	☐	***34-39***
12.3 Does the protocol address other limitations?	☒	☐	☐	***44-45***

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?	☒	☐	☐	***48***
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☒	☐	
13.3 Have data protection requirements been described?	☒	☐	☐	***39***

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?	☒	☐	☐	***20***

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)?	☒	☐	☐	***48***
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	***48***

Comments:

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

Date: / /

***Signature:* _____**

(Administrative Change 1, 24 September 2021)

Statistical Analysis Plan Amendment 1

Study ID: 205071

Official Title of Study: A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the Plasmodium falciparum parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age

Date of Document: 08-October-2020

Information Type:	Statistical Analysis Plan (SAP)

TITLE PAGE

Protocol Title:	A phase IV, longitudinal, cross-sectional, retrospective, ancillary epidemiology study of the EPI-MAL-005 study to evaluate the genetic diversity in the *Plasmodium falciparum* parasite circumsporozoite sequences before and after the implementation of the RTS,S/AS01$_E$ vaccine in malaria-positive subjects ranging from 6 months to less than 5 years of age.
Study Number:	205071
Compound Number:	SB257049
Abbreviated Title:	EPI-MALARIA-010 VS AME

Sponsor Name: GlaxoSmithKline Biologicals SA (GSK)

Regulatory Agency Identifier Number(s): Not Applicable

Registry	**ID**
EU PAS Register No	**EUPAS42948**

Statistical Analysis Plan (SAP) Template v3.0 14 September 2022

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VERSION HISTORY

SAP Version	Approval Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
SAP	18 January 2024	08 October 2020	Not Applicable	Original version
SAP Amendment 1	10 Dec 2024	08 October 2020	Clarifications on the definition of prevalence Addition of logistic piecewise models and amendment of the trend analysis section to address EMA questions (procedure EMEA/H/W/002300/MEA/015.2)	Answer RTQ, clarifications

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to describe the planned statistical analyses for Study EPI-MALARIA-010 VS AME (205071). Details of the planned interim analysis, as well as the final analyses, are provided.

1.1. Objectives, Estimands and Endpoints

Objectives	Endpoints
Primary	
• To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.	• Occurrence of specific *P. falciparum* haplotype infection at subject level.
• To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$.	• Occurrence of specific *P. falciparum* haplotype infection at haplotypes level.
Secondary	
• To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$ vaccination status.	• Occurrence of specific *P. falciparum* haplotype infection at subject level by sub-groups.
• To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.	• Occurrence of specific *P. falciparum* haplotype infection at haplotypes level by sub-groups.
• To estimate trends in longitudinal prevalence of *P. falciparum* in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$.	• Estimate trends of specific *P. falciparum* haplotype infection at subject level by RTS,S/AS01$_E$ vaccination status.
• To estimate trends in longitudinal frequency of *P. falciparum* in subjects aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.	• Estimate trends of specific *P. falciparum* haplotype infection at haplotype level by RTS,S/AS01$_E$ vaccination status.

Primary estimand

The primary question of interest is: Could we characterise *P. falciparum* haplotypes detected in young children aged 6 months to <5 years over time before and after the introduction of the RTS,S/AS01$_E$ vaccine on one site in Eastern Africa and one site in Western Africa, based on samples collected in the framework of the EPI-MALARIA-005 study.

The estimand is described by the following attributes:

- *Population:* children aged 6 months to <5 years.
- *Biological samples:* re-use DNA samples collected and processed during the EPI-MALARIA-005 study.
- *Variable / endpoint:* frequency and prevalence of 3D7 haplotype (strain included in the RTS,S vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.

1.2. Study Design

Overview of Study Design and Key Features	
Design Features	• Longitudinal, retrospective, epidemiological, cross-sectional study, ancillary to the EPI-MALARIA-005 study. In order to characterise *P. falciparum* haplotypes, genotyping will be conducted on samples of subjects aged 6 months to <5 years with *P. falciparum* infection, collected in the framework of the EPI-MALARIA-005 study at two sites before and after the start of RTS,S/AS01$_E$ vaccination, one site in Eastern Africa and one site in Western Africa. Those sites will be the same during the entire duration of the study. • Study population: Subjects aged 6 months to <5 years of age, enrolled in the EPI-MALARIA-005 study at the two sites before and after the start of RTS,S/AS01$_E$ vaccination, may be included in the EPI-MALARIA-010 study. • Biological samples: In the EPI-MALARIA-005 study, a blood sample is obtained for malaria blood slide reading conducted locally and 2 to 3 drops of blood are spotted onto filter paper for the Nucleic Acid Amplification Test (NAAT) (conducted at AMC [Academic Medical Centre], Amsterdam, The Netherlands). Both blood slide reading (by microscopy) and NAAT (from genomic deoxyribonucleic acid [DNA]) will be used to evaluate the level of asexual *P. falciparum* parasitaemia. The EPI-MALARIA-010 study will re-use DNA samples collected and processed during the EPI-MALARIA-005 study. For samples that are identified as positive for *P. falciparum* by malaria blood slide reading and/or by NAAT, a minimum of 15 microliters of DNA extracted at AMC will be sent to HSPH/BI for amplicon sequencing. • Sampling schedule: The EPI-MALARIA-010 study will re-use samples from subjects enrolled in 7 consecutive annual cross-sectional surveys of the EPI-MALARIA-005 study. Selection of surveys included in EPI-MALARIA-010 is contingent upon the start date of the MVIP and will require the first surveys to occur prior to MVIP start. • Primary completion date (PCD): PCD is defined as the date of final collection of data for all primary outcomes. • End of study (EoS): Last testing results released of samples re-used from EPI-MALARIA-005. EoS must be achieved no later than 8 months after the selection of the samples for testing of the last survey.

Overview of Study Design and Key Features	
	• Duration of the study: This design allows monitoring the yearly variability of *P. falciparum* haplotype frequency and prevalence before and after the start of RTS,S/AS01$_E$ vaccination, across 7 consecutive annual surveys as described below: – Epoch 001: Survey 3 from EPI-MALARIA-005 study – Epoch 002: Survey 4 from EPI-MALARIA-005 study – Epoch 003: Survey 5 from EPI-MALARIA-005 study – Epoch 004: Survey 6 from EPI-MALARIA-005 study – Epoch 005: Survey 7 from EPI-MALARIA-005 study – Epoch 006: Survey 8 from EPI-MALARIA-005 study – Epoch 007: Survey 9 from EPI-MALARIA-005 study
Study intervention	• Not applicable: Observational study
Study intervention Assignment	• Not applicable: Observational study
Interim Analysis	• An interim analysis will be performed from the 3 surveys before vaccination implementation and the first 2 surveys after vaccination implementation. • As the analyses are based on Survey year and sites, at the time of the Interim analysis we will compute prevalence and frequency of haplotypes objectives on Survey 3 to 7 from EPI-MALARIA-005 study. • The complete list of tables, listings and figures performed at the Interim Analysis will be notify in the Output and Programming Specification (OPS) associated document.

2. STATISTICAL HYPOTHESES

Not applicable. All analyses are descriptive in nature.

2.1. Multiplicity Adjustment

Not applicable.

3. ANALYSIS SETS

Analysis Set	Definition / Criteria	Analyses Evaluated
Analysis Set (AS)	Study participants: • aged **6 months to <5 years** enrolled in the EPI-MALARIA-005 study at **Kintampo** in Ghana (Western Africa) or **Kombewa** in Kenya (Eastern Africa), **fulfilling inclusion and exclusion criteria** of the EPI-MALARIA-005 study. • whose parent(s)/ Legally Acceptable Representative(s) [LAR(s)] have provided **informed consent** for the use of collected blood samples and data in **further research** as explained in the original informed consent form (ICF) of the EPI-MALARIA-005 study. • recruited from Survey 3 to Survey 9.	• Study Population

4. STATISTICAL ANALYSES

4.1. General Considerations

SAS version 9.4 or higher version will be used for statistical analysis.

All analyses will be conducted on the Analysis set (AS). Unless specified otherwise, all the statistical tests will be two-sided at alpha level of 0.05.

4.1.1. General Methodology

- Continuous data will be summarised using descriptive statistics: n, mean, standard deviation (SD), median, minimum and maximum, and if required 25^{th} and 75^{th} percentiles.
  Categorical data will be summarised as the number and percentage of each category.
- The asymptotic 95% confidence interval (CI) for a mean within a group will be calculated. And the 95% CI for proportion will be based on exact Clopper-Pearson confidence interval [Clopper, 1934].

4.1.2. Study groups

The following sub-groups will be used for the statistical analyses:

Table 1 Study groups

Group order in tables	Group label in tables	Group definition for footnote
Age group (according WHO definition [years])		
1	0.5-1Y	6 months – 1 year at ICF date
2	2-4Y	2 – 4 years at ICF date
Gender		
1	Female	Female
2	Male	Male
RTS,S/AS01$_E$ vaccination status		
1	Vaccinated	Children vaccinated with RTS,S/AS01$_E$ malaria vaccine*
2	Unvaccinated	Children unvaccinated with RTS,S/AS01$_E$ malaria vaccine*
Co-infection		
1	No	Children infected with ≤1 haplotype
2	Yes	Children infected with >1 haplotype

* see Section 6.2.1.2

Outputs will be displayed by site according to Survey.

Table 2 Sites

Site n°	Vaccine exposed status	Group label used in tables	Country	Districts (eCRF denomination - Demography)
Study site				
207083	Exposed	207083_GH_Kintampo_ EXP	Ghana	Kintampo North, Kintampo South, Nkoranza North and Techiman North
207082	Exposed	207082_KE_Kombewa	Kenya	Kombewa

4.1.3. Sequence of analysis

An *interim analysis* will be performed using from the 3 surveys before vaccination implementation and the first 2 surveys after vaccination implementation. The interim analysis will only be descriptive.

The *final analysis* will be performed using data from the 2 last surveys.

Trends analysis related to secondary objectives will be performed only when all data will be available, thus at the time of the final analysis.

As the analyses are based on Survey year and sites the following objectives will be assessed during interim and final analyses:

Table 3 Sequence of analyses

Interim/Final analysis	Objectives	Surveys
Interim Analysis	**Primary objectives** • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$ • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_{E.}$ **Secondary objectives** • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$vaccination status. • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.	Survey 3 to Survey 7
Final Analysis	**Primary objectives** • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$. • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$. **Secondary objectives** • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype prevalence in subjects aged 6 months to <5 years by age group, gender and RTS,S/AS01$_E$vaccination status. • To obtain study site-specific longitudinal estimates of *P. falciparum* haplotype frequency in subjects aged 6 months to	Survey 8 to Survey 9

Interim/Final analysis	Objectives	Surveys
	<5 years with *P. falciparum* infection by age group, gender and RTS,S/AS01$_E$ vaccination status.	
Final Analysis	**Secondary objectives** • To estimate trends in longitudinal prevalence of *P. falciparum* in subjects aged 6 months to <5 years vaccinated or not with RTS,S/AS01$_E$. • To estimate trends in longitudinal frequency of *P. falciparum* in subjects aged 6 months to <5 years with *P. falciparum* infection vaccinated or not with RTS,S/AS01$_E$.	Survey 3 to Survey 9

The complete list of tables, listings and figures is provided in the Output and Programming Specification (OPS) associated document.

4.1.4. Baseline Definition

4.1.4.1. Haplotype prevalence

The **haplotype prevalence** corresponds to the number of subjects infected with a specific *P. falciparum* haplotype at a given amplicon, divided by the total number of subjects.

Table 4 Haplotype Prevalence

$$H_i\ Prevalence = \frac{number\ of\ subjects\ infected\ with\ H_i}{Total\ number\ of\ subjects}$$

Thus, the denominator will be all the subjects aged 6 months to <5 years included in the EPI-MALARIA-010 study for each of the two sites considered and at each survey while the numerator is based on results of haplotype detected on malaria positive subjects extracted from EPI-MALARIA-005 study.

4.1.4.2. Haplotype frequency

The **haplotype frequency** is defined as the number of occurrences of a specific *P. falciparum* haplotype in the population, divided by the total number of haplotypes detected in the population.

Table 5 Haplotype frequency

$$H_i\ frequency = \frac{number\ of\ infection\ with\ H_i}{Total\ number\ haplotypes\ detected}$$

Thus, in case of co-occurring infections with *P. falciparum* malaria, the same sample will contribute multiple times in the denominator.

Note that the frequency will be estimated using data only from malaria positive subjects aged 6 months to <5 years included in the EPI-MALARIA-010 study for each of the two sites considered.

4.1.4.3. Study Population

Subjects aged **6 months to <5 years of age**, initially enrolled in the EPI-MALARIA-005 study at **Kintampo** in Ghana (Western Africa) or **Kombewa** in Kenya (Eastern Africa) before and after the start of RTS,S/AS01$_E$ vaccination.

These sites are located in the planned RTS,S/AS01$_E$ pilot implementation areas of Ghana and Kenya, which are moderate-to-high transmission areas as recommended by the World Health Organization (WHO) for the Malaria Vaccine Implementation Programme (MVIP).

The study participants must satisfy all the following criteria at study entry:

- **fulfilling inclusion and exclusion criteria** of the EPI-MALARIA-005 study.
- whose parent(s)/ Legally Acceptable Representative(s) [LAR(s)] have provided **informed consent** for the use of collected blood samples and data in **further research** as explained in the original informed consent form (ICF) of the EPI-MALARIA-005 study.
- recruited from **Survey 3 to Survey 9** on EPI-MALARIA-005 study.

The EPI-MALARIA-010 study will re-use DNA samples collected and processed during the EPI-MALARIA-005 study.

Only for samples that are identified as **positive** for ***P. falciparum*** **infection** obtained by malaria blood slide reading and/or NAAT detected in the EPI-MALARIA-005 study, a minimum of 15 microliters of DNA extracted at AMC will be sent to Harvard T. H. Chan School of Public Health (HSPH)/ Broad Institute (BI) for amplicon sequencing.

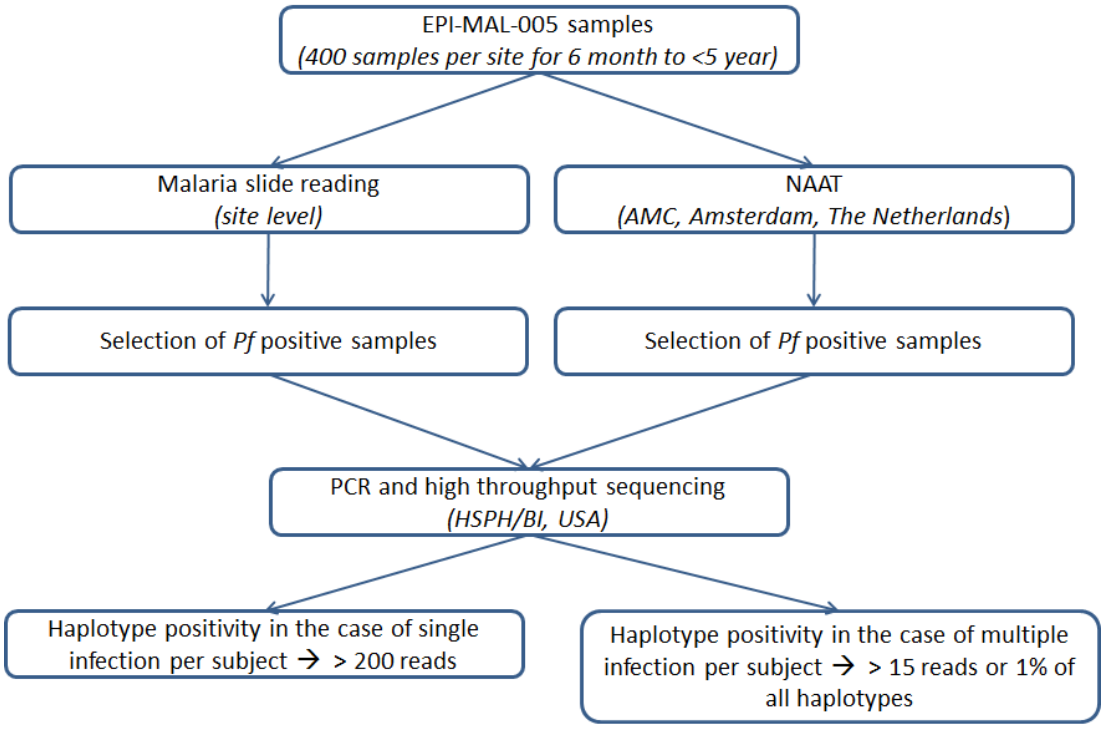
4.1.4.4. Biological sample

Amplicon Sequencing Approach

For subjects identified as positive for malaria by blood slide reading and/or NAAT analysis, a minimum of 15 µl of DNA/sample extracted at AMC will then be sent to HSPH/BI in pre-barcoded sample tubes to be supplied by the BI. Custom workflows developed at the BI will be used to PCR amplify target regions of the DNA in each sample, which will then be sequenced using the Illumina MiSeq platform. This process is referred to amplicon sequencing.

The selection of samples and order of tests to be performed at the different locations is indicated in Figure 1.

Figure 1 Selection of samples and sequence of tests to be performed



NAAT = Nucleic Acid Amplification Test; AMC = Academic Medical Centre; *Pf* = *Plasmodium falciparum;* HSPH = Harvard T.H. Chan School of Public Health; BI = Broad Institute.

Parasite positivity is defined as samples returning > 200 sequencing reads mapping to at least one amplicon as performed at the HSPH/BI for single infections.

Per EPI-MALARIA-010 protocol, in the case of multiple infections samples, the **threshold** for calling an individual **haplotype** within a parasite positive sample (which contains many haplotypes) is **15 reads or 1% of all haplotypes from the same subject**.

However, initial optimistic haplotype threshold of 15 reads defined in the protocol originated from MALARIA-066 study where positive samples were rich with parasite genetic materials.

After analysing more recent data generated in preparation for MALARIA-095 study and from the MALARIA-095 study itself, a new threshold point of 50 reads is considered more accurate. MALARIA-010 and MALARIA-095 studies share a more updated sequencing protocol relative to MALARIA-066 study, which makes the comparison to MALARIA-095 study more relevant.

Furthermore, a sensitivity analysis on a threshold of 50 reads will be provided.

For the analysis, HSPH/BI will generate PCR-generated amplicon data that will be sequenced on an Illumina MiSeq. PCR amplicons will target two *P. falciparum* loci: a portion of the CSP C-terminus that overlaps the RTS,S vaccine construct (CSP) and an

equally polymorphic region of *serine repeat antigen 2* (SERA2). For each sample, haplotypes at each amplicon will be generated from the Illumina short-read data using the same pipeline employed in MALARIA-095 study. Haplotypes will be analysed at the amino acid level. We will use the **CSP results** as per the MALARIA-066 study. The SERA2 sequences could be used as a control of the CSP results for further investigations.

4.1.4.5. Handling missing data

We could have two kinds of missing data.

- A blood sample which does not reach the minimum of 15 microliters of DNA extracted at AMC.
- The PCR test could fail and not return a full length of DNA sequence that will be considered as missing data.

  Note that we expect around 15 to 20% of PCRs to fail.

For the Haplotype prevalence, a *best*- and *worst-case* allocation (refer Section 4.2.3.1 and Section 4.2.3.2) will be done using the subject level total dataset for non-evaluable samples with the sequencing method (subjects with non-evaluable *P. falciparum* haplotype).

4.2. Primary Endpoints Analyses

4.2.1. Definition of endpoints

- Occurrence of specific *P. falciparum* haplotype infection at subject level.
- Occurrence of specific *P. falciparum* haplotype infection at haplotypes level.

4.2.2. Main analytical approach

All primary analyses will be reported by site and survey.

The analyses will be performed on the 3D7 haplotype (strain included in the RTS,S vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.

The final analysis will include a table regrouping all survey data (each haplotype reported within at least one survey) by site and by Survey.

All CIs will be two-sided 95% CIs computed using the exact method [Clopper, 1934].

- Occurrence of specific *P. falciparum* haplotype infection at subject level.

The *haplotype prevalence* will be estimated as the number of subjects infected with a specific *P. falciparum* haplotype, divided by the total number of subjects (refer Section 4.1.4.1).

- Occurrence of specific *P. falciparum* haplotype infection at haplotypes level.

The *haplotype frequency* will be estimated as the number of occurrences of a specific *P. falciparum* haplotype, divided by the total number of haplotypes detected in the study population (refer Section 4.1.4.2).

4.2.3. Sensitivity analyses

4.2.3.1. Best case scenario

The primary analysis is consistent with a best case allocation since it will be performed considering all the non-evaluable samples as negative samples for the haplotype considered.

4.2.3.2. Worst case scenario

In the context of non-evaluable samples with the sequencing method, a sensitivity analyses (worst case allocation) will be performed considering all the non-evaluable samples as positive samples for the haplotype considered.

The haplotype prevalence (refer Section 4.1.4.1) will be estimated on the 3D7 haplotype (strain included in the RTS,S vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey.

In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.

The final analysis will include a table regrouping all survey data (each haplotype reported within at least one survey) by site and by Survey.

4.2.3.3. Threshold

Initial haplotype threshold of 15 reads was defined in the protocol of the EPI-MALARIA-010 study (refer Section 4.1.4.4) based on MALARIA-066 study where positive samples were rich with parasite genetic materials.

From the conclusions of other research conducted at the BI on MALARIA-066 and MALARIA-095 studies, after GSK internal scientific re-assessment, and the fact that more sterile samples (less parasite genetic material) are present in EPI-MALARIA-010 study, it appears that additional 50 reads threshold should be considered in the EPI-MALARIA-010 study.

In this context, a sensitivity analysis will be computed on a threshold of 50 reads.

The haplotype prevalence (refer Section 4.1.4.1) and frequency (refer Section 4.1.4.2) will be estimated on the 3D7 haplotype (strain included in the RTS,S vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey.

In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.

The final analysis will include a table regrouping all survey data (each haplotype reported within at least one survey) by site and by Survey.

4.3. Secondary Endpoints Analyses

4.3.1. Definition of endpoints

- Occurrence of specific *P. falciparum* haplotype infection at subject level by sub-groups.
- Occurrence of specific *P. falciparum* haplotype infection at haplotypes level by sub-groups.
- Estimate trends of specific *P. falciparum* haplotype infection at subject level by RTS,S/AS01$_E$ vaccination status.
- Estimate trends of specific *P. falciparum* haplotype infection at subject level by RTS,S/AS01$_E$ vaccination status.

4.3.2. Main analytical approach

4.3.2.1. Prevalence and frequency on sub-groups

All secondary analyses on prevalence and frequency will be reported by site and per survey.

The analyses will be performed on the 3D7 haplotype (strain included in the RTS,S vaccine) and on the haplotypes with a frequency ≥5% in the site during the survey.
In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.

The final analysis will include a table regrouping all survey data (each haplotype reported within at least one survey) by site and by Survey.

All CIs will be two-sided 95% CIs computed using the exact method [Clopper, 1934]

- Occurrence of specific *P. falciparum* haplotype infection at subject level by sub-groups.

The *haplotype prevalence* (refer Section 4.1.4.1) will be estimated by gender, age group and RTS,S vaccination status (refer Section 4.1.2).

- Occurrence of specific *P. falciparum* haplotype infection at haplotypes level by sub-groups.

The *haplotype frequency* (refer Section 4.1.4.2) will be estimated by gender, age group and RTS,S vaccination status (refer Section 4.1.2).

4.3.2.2. Trends analysis

The trends analysis will be done only on the 3D7 haplotype and the haplotypes with more than 5% of frequency across the 7 surveys and reported by site according to RTS,S vaccination status (refer Section 4.1.2).

- Estimate trends of specific *P. falciparum* haplotype infection at subject level by RTS,S/AS01$_E$ vaccination status.

Trends and annual fluctuations in haplotype prevalence will be analysed using *logistic regression*. This method will allow studying trends without making assumptions regarding annual fluctuations.

- No adjustment for multiple testing will be employed. The assumptions of the logistic regression models will be verified (multicollinearity, linearity)

Logistic models will be used to describe the nature of the relationship between the dependent variable (probability of having *P. falciparum* infection with a given haplotype) and the survey's year.

In addition, piecewise logistic regression models will be used to describe the nature of the relationship between the dependent variable (specific haplotype infection at subject level) and the survey's year. We will consider a prespecified breakpoint based on the year following vaccine introduction (Survey 7) and additionally an analysis allowing the model itself to identify a breakpoint. The goal of this approach is to provide a more flexible model that can capture the underlying patterns in the data.

- Crude odds ratio (OR) and 95% CI will be estimated using a univariable logistic regression.
- Adjusted OR and 95% CI will be estimated using a multivariable logistic regression model adjusted on gender, age groups, vaccination status and presence/absence of co-infection.

Reference category is show in the Table 6.

Table 6 Reference category for Odds ratio Analyses

Variable	Reference category
Survey	Survey 3
Gender	Female
Age group	0.5-1Y
RTS vaccination status	Vaccinated
Co-infection	No

Additional models might be explored if deemed necessary in light of the models' results and interpretation.

- Estimate trends of specific *P. falciparum* haplotype infection at haplotype level by RTS,S/AS01$_E$ vaccination status.

The haplotypes frequencies on the 3D7 haplotype and the haplotypes with more than 5% of frequency across the 7 surveys will be presented over survey time points with a bar charts according to RTS vaccination status.

4.3.3. Additional analyses

In addition of logistic regression models, we will produce line plot graphs to present the haplotype prevalence of the 3D7 haplotype and the haplotypes with more than 5% of frequency across the 7 surveys over survey time points.

All graphs will be presented by site.

4.4. Interim Analyses

An interim analysis will be performed using data from the 3 surveys before vaccination implementation and the first 2 surveys after vaccination implementation.

As the analyses are based on Survey year and sites, at the time of the Interim analysis we will compute prevalence and frequency of haplotypes objectives on Surveys 3 to 7 from the EPI-MAL-005 study.

The complete list of tables, listings and figures performed at the Interim Analysis will be available in the Output and Programming Specification (OPS) associated document.

4.5. Changes to Protocol Defined Analyses

Changes from the originally planned statistical analysis specified in the protocol are detailed in Table 7

Table 7 Changes to Protocol Defined Analysis Plan

Protocol Defined Analysis	SAP Defined Analysis	Rationale for Changes
• The numbering of surveys starts from Survey 1 to 7 in the protocol.	• We updated the numbering to Survey 3 to Survey 9 to be aligned with the numbering of the EPI-MALARIA-005 study.	• We updated the survey number to follow exactly the numbering of the EPI-MALARIA-005 study to facilitate the comparison between both studies.
• Clones	• The term “clones” that was often used in protocol, will be replaced in the SAP by the term “haplotypes”	• We will use haplotypes instead of clones because we didn’t receive information about details of clones in the lab data and in our study. Haplotype is the most relevant analysis level for the study objectives.
• The analyses were planned on all	• In the SAP, we restrict the analyses on the 3D7 strain and the haplotypes	• Due to the large number of haplotypes that could be detected

Protocol Defined Analysis	SAP Defined Analysis	Rationale for Changes
haplotypes in the protocol.	with a frequency ≥5% in the site during the survey. In case of less than 10 haplotypes with a frequency ≥5%, we will present the 10 most frequent haplotypes detected.	we decided to focus only on more frequent haplotypes.
• Contrast analysis planned to evaluate trends	• We will replace the contrast analysis by producing graphs on prevalence/frequency over survey time.	• The contrast analysis implies a too large number of possible choices of contrast to be tested, as there is no expectation of trends (linear, cubic, quadratic, …).
• A multinomial logistic regression to estimate trends on haplotype frequency	• We will replace the multinomial logistic regression by producing graphs on prevalence/frequency over survey time.	• A multinomial logistic regression is not applicable in our frequency trends analysis. There are a very large number of distinct haplotypes detected in the population. It means that it is not possible to evaluate which one could be correlated. In addition, we have in our study a maximum of 7 points to be analysed (7 surveys) with the possibility that a haplotype will not be detected during one of the survey with the consequence that it would be removed from the model. In conclusion, multinomial logistic regression will not be appropriate.
• Analysis planned on threshold of 15 reads	• An additional sensitivity analysis will be performed on a threshold of 50 reads	• Due to more sterile samples, threshold was reassessed and additional threshold of 50 reads will be analysed.

5. SAMPLE SIZE DETERMINATION

5.1.1. Study size considerations

The sample size is per design limited to the number of subjects included in the EPI-MALARIA-005 study. All eligible children below 5 years of age enrolled in EPI-MALARIA-005 during 7 cross-sectional surveys may be included in EPI-MALARIA-010 regardless of their parasitaemia status. Hence, 400 children below 5 years of age are expected to be enrolled every year in each of the two EPI-MALARIA-010 study sites.

Malaria prevalence was estimated at 25% for children aged 6 months to 5 years in the selected sites based on GSK sponsored studies in the same centres (data from the EPI-MALARIA-005 study had its first survey collected in 2014-2015).

Depending on the variations in parasite prevalence over the years, around 100 subjects are expected to have a positive malaria parasitaemia per site and per year.

5.1.2. Power considerations for prevalence analyses

Prevalence will be analysed at subject level, the sample size is defined as the total number of subjects per year and per study site. As described in Section 5.1.1, 400 subjects are expected to be included per site and per year.

The power for the prevalence analysis is computed using a sample size of 400 subjects per site and per year.

5.1.2.1. Prevalence estimates' precision

The expected precision around the estimation of the haplotype prevalence is presented in this section for a prevalence range of 0.5% to 10%. The range of 0.5% to 10% prevalence was chosen based on the prevalence of the 3D7 haplotype observed in the MALARIA-066 study and to cover scenarios involving more frequent haplotypes.

The expected range of the CI around different values of haplotype prevalence is computed in Table 8 considering a sample size of 400 children per site and per survey (all children with positive and negative parasitaemia are considered to compute the prevalence, see prevalence in Table 8). CI limits are computed using the exact 95% CI.

Table 8 Exact 95% CI limits computed around different values of observed haplotype prevalence

Sample size	Exact	Expected haplotype prevalence			
	95% CI	0.5%	1%	5%	10%
400	LL (%)	0.1%	0.3%	3.1%	7.2%
	UL (%)	1.8%	2.5%	7.6%	13.4%

LL: Lower Limit; UL Upper Limit
Exact 95% CI limits computed with SAS 9.2.

5.1.2.2. Power considerations for prevalence trends analyses

Trends in longitudinal prevalence of *P. falciparum* haplotypes in subjects aged 6 months to <5 years, vaccinated or not with RTS,S/AS01$_E$, will be assessed by logistic model.

Power computations are based on the logistic model contrast analysing the variation in prevalence before and after vaccination implementation.

Power is computed based on a scenario with a total of 7 surveys, divided into 3 surveys before and 4 surveys after vaccination implementation.

The following parameters, mainly based on MALARIA-066 published and unpublished data, are also taken into account in the power computation:

- Proportions for prevalence trends analysis ranging from 0.5% to 10% (as described in Section 5.1.2.1).

- A 30% to 60% increase/decrease in prevalence before versus after vaccination implementation (on an indicative basis, the incidence of the 3D7 haplotype showed a 50% decrease after vaccination in MALARIA-066).

Power is computed for adjusted logistic regression models using the assumptions listed above, an alpha at 5% and an R-Square covariates parameter (R^2=0.2) to take into account the adjustment. Power computations for decreasing and increasing trends use similar assumptions.

Power computations are presented in Table 9 and Table 10 below, for decreasing trend and for increasing trend, respectively.

Table 9 Power to detect a range of decrease in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site

	Baseline haplotype prevalence			
Expected relative decrease*	**0.5%**	**1%**	**5%**	**10%**
30%	8%	12%	42%	72%
40%	12%	20%	68%	93%
50%	18%	29%	87%	99%
60%	25%	42%	97%	>99%

* Expected relative decrease between prevalence before vs after vaccination implementation.

Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 87% to detect a relative prevalence decrease of 50% for haplotypes with a baseline prevalence of 5%, using a logistic regression analysis with an alpha of 5%.

Table 10 Power to detect a range of increase in the prevalence before and after vaccination (from 30% to 60%) depending on different baseline haplotype prevalence (from 0.5% to 10%) with a sample of 400 subjects per survey and per site

	Baseline haplotype prevalence			
Expected relative increase*	**0.5%**	**1%**	**5%**	**10%**
30%	6%	9%	32%	59%
40%	8%	13%	50%	82%
50%	10%	17%	67%	94%
60%	13%	22%	81%	99%

* Expected relative increase between prevalence before vs after vaccination implementation.

Power computed using PASS 12 for the logistic model contrast analysing prevalence before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 81% to detect a relative prevalence increase of 60% for haplotypes with a baseline prevalence of 5%, using a logistic regression analysis with an alpha of 5%.

5.1.3. Power considerations for frequency analyses

Frequency will be analysed at haplotype level, the sample size is defined as the total number of haplotypes detected per year and per study site.

The number of detected haplotypes expected per site and per year is based on the prevalence observed in the selected sites in the first year of EPI-MALARIA-005, and on the number of haplotypes observed by subject in MALARIA-066 [Neafsey, 2015].

Assuming an average CoI of 3 haplotypes per subject with positive parasitaemia, as computed based on MALARIA-066 data, and a malaria prevalence of 25% in both study sites as observed in EPI-MALARIA-005, 300 haplotypes for 100 malaria positive subjects per site and per year are expected.

The power for frequency analysis is computed using a sample size of 300 haplotypes per site and per year.

5.1.3.1. Frequency estimates' precision

The expected precision around the estimation of the haplotype frequency is presented in this section for a frequency range of 1% to 20%. Given the limited availability of frequency data for haplotypes other than 3D7, the range 1% to 20% was chosen to cover scenarios involving a range of rare and more frequent haplotypes.

The expected range of the CI around different values of haplotype frequency is computed in Table 11 considering a sample size of 300 haplotypes, assuming an average CoI of 3 haplotypes per subject as observed in the MALARIA-066 study, for 100 subjects with positive parasitaemia per site and per survey. CI limits are computed using the exact 95% CI.

Table 11 Exact 95% CI limits computed around different values of observed haplotype frequency

Sample size (haplotypes)	Exact 95% CI	Expected haplotype frequency				
		1%	5%	10%	15%	20%
300	LL (%)	0.2%	2.8%	6.8%	11.2%	15.6%
	UL (%)	2.9%	8.1%	14.0%	19.6%	25.0%

LL: Lower Limit; UL Upper Limit
Exact 95% CI limits computed with SAS 9.2.

5.1.3.2. Power considerations for frequency trends analyses

Trends in longitudinal frequency of *P. falciparum* haplotypes in subjects aged 6 months to <5 years with *P. falciparum* infection, vaccinated or not with RTS,S/AS01$_E$, will be assessed by logistic model.

Power considerations for frequency trends analyses are computed using the same method as for the prevalence trends analyses.

Power computations are based on the logistic model contrast analysing the variation in frequency before and after vaccination implementation.

Power is computed based on a scenario with a total of 7 surveys, divided into 3 surveys before and 4 surveys after vaccination implementation.

The following parameters, mainly based on MALARIA-066 published and unpublished data, are also taken into account in the power computation:

- Proportions for frequency trends analysis ranging from 1% to 20% (as described in Section 5.1.3.1).
- A 30% to 60% increase/decrease in frequency before versus after vaccination implementation (on an indicative basis, the incidence of the 3D7 haplotype showed a 50% decrease after vaccination in MALARIA-066).

Power is computed for adjusted logistic regression models using the assumptions listed above, an alpha at 5% and an R-Square covariates parameter (R^2=0.2) to take into account the adjustment. Power computations for decreasing and increasing trends use similar assumptions.

Power computations are presented in Table 12 and Table 13 below, for decreasing trend and for increasing trend, respectively.

Table 12 Power to detect a range of decrease in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 haplotypes per survey and per site

	Baseline haplotype frequency			
Expected relative decrease*	**1%**	**5%**	**10%**	**20%**
30%	10%	34%	60%	90%
40%	16%	56%	85%	99%
50%	24%	77%	97%	>99%
60%	33%	92%	>99%	>99%

* Expected relative decrease between frequency before vs after vaccination implementation.

Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 92% to detect a relative frequency decrease of 60% for haplotypes with a baseline frequency of 5%, using a logistic regression analysis with an alpha of 5%.

Table 13 Power to detect a range of increase in the frequency before and after vaccination (from 30% to 60%) depending on different baseline haplotype frequency (from 1% to 20%) with a sample of 300 haplotypes per survey and per site

	Baseline haplotype frequency			
Expected relative increase*	**1%**	**5%**	**10%**	**20%**
30%	8%	25%	47%	82%
40%	11%	39%	70%	97%
50%	14%	54%	86%	>99%
60%	18%	69%	95%	>99%

* Expected relative increase between frequency before vs after vaccination implementation.

Power computed using PASS 12 for the logistic model contrast analysing frequency before vs after vaccination implementation, alpha = 0.05, R-square covariates = 0.2.

The power reaches 86% to detect a relative frequency increase of 50% for haplotypes with a baseline frequency of 10%, using a logistic regression analysis with an alpha of 5%.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Study Population Analyses

6.1.1. Participant Disposition

A summary of the number of participants by sites and per survey will be provided. The table will also include counts of invalid sample results per site and survey.

6.1.2. Demographic and Baseline Characteristics

The demographic characteristics including age group (refer Section 4.1.2), gender and the RTS,S vaccination status (refer Section 6.2.1.2) will be summarized with descriptive statistics by site and per survey.

The RTS,S vaccination (number of doses) will be also summarized by age group.

6.1.3. Protocol Deviations

The list of samples with quantity not sufficient (less than 15 µl of DNA/sample) sent to Broad Institute from AMC will be provided in the Protocol Deviation sheet.

Note that in some case, BI would be able to analyse the sample.

6.2. Appendix 2 Data Derivations Rule

6.2.1. Demographic data

6.2.1.1. Age at Inform consent in years

The age will be computed in years as the difference between the informed consent date and the date of birth divided by 365.25 or taken directly as reported in the CRF if the date of birth is unavailable.

The age will be categorized according to WHO definition: 0.5-1, 2-4 years.

6.2.1.2. Definition of RTS,S/ $AS01_E$ vaccination status

The **vaccinated group** is defined as those subjects who received at least one dose of the RTS,S/$AS01_E$ vaccine.

The **unvaccinated group** is defined as those subjects who received no dose of the RTS,S/$AS01_E$ vaccine.

The vaccination status will be determined only for the surveys/centres starting after the introduction of the vaccine.

Note: some vaccinated subjects were able to take part in clinical studies of the RTS,S/$AS01_E$ vaccine during phase III, so these subjects were vaccinated before the introduction of the vaccine.

6.2.2. Display of decimals

6.2.2.1. Percentages

Percentages and their corresponding confidence limits will be displayed with one decimal except for 100% and a real 0% (no case) in which case no decimal will be displayed.

In the case of one decimal should not be explicative (e.g. 0.0%), the standard rounding rule describe in the Table 14 will be apply.

Table 14 Standard rounding rule for decimals

n/N	Displayed percentage
1/3000	0.03%
1/30000	0.003%
299/300	99.7%
2999/3000	99.97%
29999/30000	99.997%

6.2.2.2. Continuous data

The following decimal description will be used for the analyses :

Parameters	Number of decimal digits
Minimum, maximum, range	Number of decimals in the raw data
Mean, median	Number of decimals in the raw data + 1
Standard deviation	Number of decimals in the raw data + 2

6.2.3. Handling of Partial Dates

<table>
<tr><th>Element</th><th>Reporting Detail</th></tr>
<tr><td>Age at Inform consent</td><td>
Partial dates to compute the age at Inform consent recorded in the CRF will be imputed using the following convention:
<table>
<tr><td>Missing day and month</td><td>As only the year available for the date of birth, we will impute missing day and months to 30th June of the year to compute the age at Inform consent.</td></tr>
<tr><td>Missing day</td><td>We will impute missing day as 15th of the month.</td></tr>
</table>
In the case of the imputed age of the participant is less than 6 months but the eligibility criteria are met, we will impute the age at 6 months old.
</td></tr>
</table>

6.2.4. Trademarks

Trademarks of the [GlaxoSmithKline / ViiV Healthcare] Group of Companies
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6.3. Appendix 3: Abbreviations and Glossary of terms

6.3.1. List of Abbreviations

AMC	Academic Medical Centre (Amsterdam, The Netherlands)
AS	Analysis Set
AS01$_E$	GSK's proprietary Adjuvant System containing MPL, QS-21 and liposome
BI	Broad Institute
CI	Confidence Interval
CSP	CircumSporozoite Protein
DNA	DeoxyriboNucleic Acid
EoS	End of Study
EPI	Expanded Programme on Immunization
GSK	GlaxoSmithKline
HSPH	Harvard T. H. Chan School of Public Health
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
LAR	Legally Acceptable Representative
LSLV	Last Subject Last Visit
MVIP	Malaria Vaccine Implementation Programme
NAAT	Nucleic Acid Amplification Test
OPS	Output and Programming Specification

OR	Odds Ratio
PASS	Post-Authorisation Safety Study
PCD	Primary Completion Date
PCR	Polymerase Chain Reaction
P. falciparum	*Plasmodium falciparum*
RNA	RiboNucleic Acid
RTS	Hybrid protein comprising HBs (hepatitis B surface antigen) and CSP portions
RTS,S	Particulate antigen, containing both RTS and HBs antigen (S) proteins
SAP	Statistical Analysis Plan
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
WHO	World Health Organization

6.3.2. Glossary of terms

Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
End of Study: **(Synonym of End of Trial)**	For studies without collection of Human Biologicals Samples or imaging data EoS is the Last Subject Last Visit (LSLV). For database studies, EoS is the date the database analysis is completed. For studies with collection/re-use of Human Biologicals Samples or imaging data, EoS is defined as the date of the last testing/reading released of the Human Biological Samples or imaging data, related to primary and secondary endpoints. EoS must be achieved no later than 8 months after the start of the testing of the last survey
Epidemiological study:	An observational or interventional study without administration of medicinal product(s) as described in a research protocol.

Haplotype:	A haplotype is defined as a specific sequence of amino acids (AAs) at a defined amplicon locus. Two haplotypes or variants differ by at least one single nucleotide polymorphism (SNP) that generates an AA mutation in the DNA sequence (referred to as a "non-synonymous" mutation).
Non-interventional (observational) Human Subject Research:	Studies where medicinal products, should they be administered, are prescribed in normal (routine) medical practice. No medical care or medical/scientific procedures as required in a research protocol are administered to participants except as part of routine medical care.
Nucleic Acid Amplification Test (NAAT):	Although not yet considered as the gold standard, NAAT-based 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.
Post-Authorisation Safety Study:	A pharmaco-epidemiological study or a clinical trial carried out in accordance with the terms of the marketing authorisation, conducted with the aim of identifying or quantifying a safety hazard relating to an authorised medicinal product. This includes all GSK sponsored non-interventional studies and clinical trials conducted anywhere in the world that are in accordance with the terms of the European marketing authorisation and where the investigation of safety is the specific stated objective. Note: The phrase 'In accordance with the terms of the European marketing authorisation' means that the product is used according to the European label (e.g., within the recommended dose range, the approved formulation, indication etc.).

Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for all primary outcomes, whether the trial was concluded according to the pre-specified protocol or was terminated. For database studies, PCD is the date of final collection of data for all primary outcomes, whether the trial was concluded according to the pre-specified protocol or was terminated.
Prospective study:	A study in which the subjects/cases are identified and then followed forward in time in order to address one or more study objectives.
Study population:	Sample of population of interest.

7. REFERENCES

Internal documents:

Protocol :
EPI-MALARIA-010 VS AME (205071) - Protocol

EPI-MALARIA-005 study:
EPI-MALARIA-005 BOD AME (116682) - SAP Amendment 2

EPI-MALARIA-005 BOD AME (116682) - CRF annotated

Publications:

Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. Biometrika. 1934;26:404-413

Neafsey DE, Juraska M, Bedford T et al. Genetic diversity and protective efficacy of the RTS,S/AS01 Malaria vaccine. N Eng J Med. 2015; 373: 2025-37.