Supplementing Maternal and Infant Diet With Micronutrient Fortified Lipid-based Nutrient Supplements (LNS) (iLiNS-DYAD-M)

Statistical Analysis Plan

Appendix 04: Analyses on malaria immunity (version 03.0, modified on 12.02.2017)

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Contents

1. Version history

Version number	Version date	Prepared by	Description of the completed editions
01.0	24.10.2013	Upeksha Chandrasiri Prof. Stephen Rogerson	Original appendix 04
02.0	17.11.2014	Upeksha Chandrasiri Prof. Stephen Rogerson	Updated Appendix 04 to cover malaria immunity at 6 months. Additional objectives, methods, tables and figures highlighted.
03.0	12/02/2017	Prof. Stephen Rogerson	Updated Appendix 04 to cover malaria immunity at 18 months. Additional objectives, methods, tables and figures highlighted.

2. Introduction

Malaria places nearly 125 million pregnancies at risk with almost 30 million of them occurring in malaria endemic African countries alone. Malaria in pregnancy leads to over 10,000 maternal and 200,000 infant deaths each year as a result of adverse pregnancy outcomes; severe maternal anaemia, low birth weight infants, small for gestational age, still births and preterm delivery. This significant susceptibility could be attributable to the lack of acquired immunity to malaria that provides protection against clinical disease.

In addition, pregnant women residing in malaria high prevalent regions often suffer from undernourishment adding extra burden on maternal and child morbidity and mortality. Certain nutrient deficiencies such as protein energy malnutrition (PEM), Vitamin A and Zinc are known to increase susceptibility to malaria infection, possibly via suppression of the immune system. Therefore an additional supply of essential nutrients during this critical time, particularly among women living in disadvantaged families with limited resources may benefit leading to improved pregnancy or birth outcomes, child growth outcomes and even malaria clinical outcomes.

Our study is a subproject of the iLiNS-DYAD Malawi clinical trial investigating the influence of a lipid based nutrient supplementation (LNS) on the acquisition of antibody immunity to malaria in pregnant women and their children. Total IgG and functional IgG to a range of malaria antigens expressed during the asexual blood stage of the parasite; merozoite antigens (expressed on the surface and rhoptry bodies of merozoites), variant surface antigens (VSA, expressed on the surface of infected red blood cells) and schizont extract antigens were measured in plasma samples collected at enrolment (≤ 20 gestation weeks) and at 36 gestation weeks (gw) for the mothers and at 6 months and 18 months for their children.

This appendix describes the approaches taken for the statistical analyses for determining the influence of maternal nutrient supplementation on malaria antibody immunity during pregnancy and

at 6 and 18 months in infants, prepared by the iLiNS sub-contract investigators at the University of Melbourne.

3. Hypotheses to be tested

The primary hypotheses of the study are detailed as following.

Hypothesis 1: Lipid based nutrient supplementation during pregnancy increases antibody responses to malaria at 36 gw compared to multiple micronutrient supplementation (MMN) and iron and folic acid supplements (IFA)

Hypothesis 2: Maternal LNS supplementation increases malaria antibody responses in children at 6 months

Hypothesis 3: LNS supplementation during pregnancy and lactation increases antibody acquisition in children at 6 months of age compared to children of the mothers who received MMN or IFA.

Hypothesis 4: LNS supplementation during pregnancy and lactation and LNS supplementation in 6-18 month old children increases antibody acquisition in children at 18 months of age compared to children of the mothers who received MMN or IFA.

4. Study objectives

The primary aim of the iLiNS-DYAD-M study is to determine the beneficial role of nutrient supplementation on child growth outcomes. The sub-contract for the current study will investigate the secondary outcomes, determining the influence of nutrient supplementation on malaria antibody immunity during pregnancy and antibody acquisition in early childhood. Further secondary outcomes include determining the association between antibody levels and pregnancy/birth outcomes, impact of maternal nutrient supplementation on malaria antibody acquisition in infants as described below.

1. Determine the effect of the type of nutrient supplementation on malaria antibody immunity at 36 weeks' gestation. (LNS, multiple micronutrient supplementation (MMN) and iron and folic acid supplements (IFA))

2. Investigate the association between malaria antibody immunity at 6 months and the influence of maternal nutrient supplementation

3. Determine the association between maternal nutrient supplementation type and malaria antibody acquisition at 6 months

We will compare seroprevalence, and relative concentrations, of antibodies to malaria antigens (variant surface antigens, merozoite antigens and schizont extract as previously described) between children in different supplementation groups; antibodies in LNS group compared to MMN, LNS compared to IFA and MMN compared to IFA

4. Determine the association between maternal nutrient supplementation type and malaria antibody acquisition at 18 months

We will compare seroprevalence, and relative concentrations, of antibodies to malaria antigens (variant surface antigens, merozoite antigens and schizont extract as previously described) between children in different supplementation groups; antibodies in LNS group compared to MMN, LNS compared to IFA and MMN compared to IFA.

5. Definition of primary outcomes

a. Malaria antibody measurements at enrolment and at 36gw

Malaria antibody levels were measured in the peripheral plasma samples collected from pregnant women at enrolment (≤20 gw) and at 36 gw. Antibodies were measured against pregnancy-specific variant surface antigens (VSA) (including VAR2CSA DBL5 antigen), non-pregnancy-specific VSA, merozoite antigens; MSP-1 19kD, MSP-2, MSP-3, Rh2A9 (PfRh2-2030), EBA-175 and schizont extract. The antibody levels were measured by fluorimetry and will be reported as fluorescence intensity (FI), or by flow cytometry and will be reported as geometric mean fluorescence intensity (MFI) as a percentage relative to the positive control.

5.2. Changes in antibody levels and magnitude of change in antibody levels from enrolment to 36gw

Changes in antibody levels measured against all malaria antigens will be compared between enrolment and at 36gw to determine crude changes in antibody levels in the current pregnancy. For additional analyses antibody levels will be divided based on their tertiles for each antigen at each time point. Antibody levels will be first sorted from lowest to the highest. The first 1/3rd of the lowest antibody levels category will be defined as low responders

In order to calculate the magnitude of change in antibody levels following formula will be used.

Magnitude of antibody level change = Antibody levels at 36gw – Antibody levels at enrolment

5.3. Rate of change in antibody levels by 36gw

The rate of change in antibody levels will be defined as following

$$\text{Rate of change in antibody levels} = \frac{\text{Magnitude of antibody level change}}{\text{Number of weeks from enrolment to 36gw}}$$

The number of weeks from enrolment to 36gw varies among women due to different gestational weeks at enrolment

5.4. Seropositivity to malaria antigens at enrolment and at 36gw

The seropositivity of each participant for each malaria antigen at a particular time point, enrolment or 36gw; will be defined as following

For merozoite antibodies and anti-VAR2CSA DBL5 antibodies measured by fluorimetry,

A plasma sample is considered seropositive if the MFI of the sample > average MFI of the negative controls + (3X standard deviation of the MFI of negative control)

For VSA antibodies measured via flow cytometry,

A plasma sample is considered seropositive if the Geometric MFI of sample > average geometric MFI of the negative controls + (2X standard deviation of the geometric MFI of negative control).

If seropositive to a particular malaria antigen at a particular time point “1” or if seronegative “0” will be reported. This information is included as a variable next to the respective antibody level variables in the database

5.5. Seroprevalence at enrolment and at 36gw

The seroprevalence will be defined as the proportion of women seropositive to a particular antigen at a particular time point. .

5.6. Seroconversion to malaria antigens by 36gw

Seroconversion to each malaria antigen will be determined and will be categorized as either positive or negative. A positive seroconversion is defined when the plasma collected from one pregnant woman at enrolment is seronegative (enrolment = 0) to a particular antigen when her plasma collected at 36gw becomes seropositive (36gw = 1) to the same antigen and vice versa for the negative seroconversion (enrolment = 1, 36gw = 0). The number of women who gained seropositivity and who lost seroconversion by 36gw will be calculated as a percentage of the total number of women.

5.7. Categorising pregnant women based on malaria infection status (effect modifiers and covariate adjustments)

Pregnant women will be categorised into infected and uninfected malaria based on the presence of parasitaemia by light microscopy (LM+ and LM-). This categorisation will be used in the analyses of adjustment for covariates and effect modifiers.

Light microscopy data will be obtained from Form 07, Q3

5.8. Antibody levels at 6 months (continuous outcome),

Antibodies to malaria were measured in plasma samples collected at 6 months. Antibodies to the same malaria schizont extract and merozoite antigens that were used in the maternal study were used; merozoite surface protein 1 (MSP-1), MSP-2, MSP-3, reticulocyte binding homologue 2A9 (Rh2A9) and erythrocyte binding antigen 175 (EBA-175). In addition we measured antibodies to 3 different parasite lines expressing different *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) protein (PfEMP-1 is the major variant surface antigen [VSA] involved in malaria pathogenesis). Measured antibody levels (in optical density [OD] for schizont and merozoite antigens and geometric mean fluorescence intensity [MFI] for VSA) were presented as a percentage of the positive control.

5.9. Antibody seropositivity at 6 months (dichotomous outcome)

A participant is considered seropositive for an antigen if the percentage of the sample's OD or MFI was greater than the sum of the average and 3 standard deviations (SD) of the percentage OD or MFI of the negative controls. A child who is seropositive for a particular antigen at 6 months will be assigned 1 and a child who is not seropositive will be assigned 0.

5.10. Antibody levels at 18 months (continuous outcome),

Antibodies to malaria will be measured in plasma samples collected at 18 months. Antibodies to the same malaria schizont extract and merozoite antigens that were used in the maternal study will be used; merozoite surface protein 1 (MSP-1), MSP-2, reticulocyte binding homologue 2A9 (Rh2A9) and erythrocyte binding antigen 175 (EBA-175). In addition, we will measure antibodies to 3 different parasite lines expressing different *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) protein (PfEMP-1 is the major variant surface antigen [VSA] involved in malaria pathogenesis). Measured antibody levels (in optical density [OD] for schizont and merozoite antigens and geometric mean fluorescence intensity [MFI] for VSA) will be presented as a percentage of the positive control.

5.11. Antibody seropositivity at 18 months (dichotomous outcome)

A participant will be considered seropositive for an antigen if the percentage of the sample's OD or MFI is greater than the sum of the average and 3 standard deviations (SD) of the percentage OD or MFI of the negative controls. A child who is seropositive for a particular antigen at 18 months will be assigned 1 and a child who is not seropositive will be assigned 0.

6. Basis of the analysis for objective 1: Intention to treat and per protocol

The basis for the analysis is the same as for the main trial.

7. Time points for analyses

All the analyses will be performed using antibody measures at enrolment and 36 gw. Measurements of covariates and effect modifiers collected during this time period will be used in the following analyses.

8. Presentation of study findings and hypothesis testing

8.1. Baseline information

Participant characteristics including demographic and basic clinical characteristics will be categorised by intervention groups as shown in table 1. The median and interquartile range for each characteristic will be tabulated unless otherwise stated. Differences in characteristics across the groups will be determined by Kruskal Wallis (non-parametric continuous variables), Chi^2 or Fisher Exact test (for categorical variables) where applicable.

Statistical differences between the groups will be reported as $p<0.05$ and 95% confidence intervals will be reported for the analyses.

8.2. Comparison of antibody levels and seroprevalence at enrolment and at 36gw among pregnant women in different intervention groups

Differences in antibody levels at enrolment and at 36gw across the 3 intervention groups will be compared by performing Kruskal Wallis test. If a significant difference was found in the antibody levels at enrolment between the intervention groups, enrolment malaria antibodies will be considered as a covariate in further analyses. To determine differences between MMN, LNS groups with IFA, Mann Whitney test will be performed (malaria antibody levels are not normally distributed). If the null-hypothesis (no change in antibody levels between intervention groups) was rejected for the above comparisons, Bonferroni correction will be performed adjusted for covariates and confounders described in sections 9.5 and 9.6 during the period from enrolment to 36gw. Scatter plot (similar to the presentation in figure 1) or box-whiskers plot will be constructed to display antibody levels in each supplementation group.

The seroprevalence for each antigen at 36gw will be compared between the 3 intervention groups by performing logistic regression (Table 2). Any statistically significant differences will be reported as $p<0.05$.

8.3. Magnitude and rate of change in antibody levels among pregnant women in different intervention groups

The magnitude and rate of antibody level change will be compared between the intervention groups by performing Kruskal Wallis test or if the above data is normally distributed one-way ANOVA will be performed. If null-hypothesis will be rejected, $p<0.05$, Bonferroni correction or Holm-Šídák method will be performed adjusted for the confounders and covariates. Magnitude and rate of antibody level change will be reported in table format (Table 3) or bar graphs with mean and standard error of the mean (SEM) (Figure 2 legend). Statistical differences will be reported as $p<0.05$ accompanied by 95% confidence interval.

8.4. Changes in seroconversion to malaria across the supplementation groups

The positive seroconversions at 36gw for each antigen across the intervention groups will be compared across the supplementation groups. Logistic regression will be performed to determine any differences between supplementation groups and data will be presented in table format similar to table 2. Statistical differences will be reported as $p<0.05$.

8.5. Comparison of malaria antibody levels and seroprevalence at 6 months between the three maternal supplementation arms

Figure 3A and 3B present antibody levels at 6 months reported as a percentage of the positive control stratified by the supplementation groups. Kruskal Wallis test will be performed to determine the differences in antibody levels between the supplementation arms.

Table 4 will present the median percentage antibody levels with interquartile range (IQR) at 6 months of age. Kruskal Wallis test will be performed to compare antibody levels across supplementation groups. Linear regression univariate analysis will be performed between LNS versus IFA, LNS versus MMN and MMN versus IFA to determine the antibody level differences between supplementation groups. Multivariate regression will be performed adjusting for confounders and effect modifiers. Coefficients and 95% confidence intervals (CI) will be reported.

Table 5 will present the number and the percentage of children with seropositivity to each malaria antigen by supplementation groups. Chi^2 test will be performed to determine the differences across the supplementation groups. A multivariate logistic regression will be performed adjusting for confounders, reporting relative risk ratios (RR) and 95% CI.

8.6. Testing the effect modification of factors interacting with the association between malaria immunity at 6 months and supplementation type

Table 6 will present the influence of effect modifiers on the association between antibody levels at 6 months and the supplementation type. Likelihood-ratio test will be performed to determine potential interactions. If the p-value for the ratio is <0.1, we will stratify antibody levels by the effect modifier and examine the effect of supplementation within each group.

8.7. Comparison of malaria antibody levels and seroprevalence at 18 months between the three maternal supplementation arms

Figure 4A and 4B will present antibody levels at 18 months reported as a percentage of the positive control stratified by the supplementation groups. Kruskal Wallis test will be performed to determine the differences in antibody levels between the supplementation arms.

Table 7 will present the median percentage antibody levels with interquartile range (IQR) at 18 months of age. Kruskal Wallis test will be performed to compare antibody levels across supplementation groups. Linear regression univariate analysis will be performed between LNS versus IFA, LNS versus MMN and MMN versus IFA to determine the antibody level differences between supplementation groups. Multivariate regression will be performed adjusting for confounders and effect modifiers. Coefficients and 95% confidence intervals (CI) will be reported.

Table 8 will present the number and the percentage of children with seropositivity to each malaria antigen by supplementation groups. Chi^2 test will be performed to determine the differences across the supplementation groups. A multivariate logistic regression will be performed adjusting for confounders, reporting relative risk ratios (RR) and 95% CI.

8.8. Testing the effect modification of factors interacting with the association between malaria immunity at 18 months and supplementation type

Table 9 will present the influence of effect modifiers on the association between antibody levels at 18 months and the supplementation type. Likelihood-ratio test will be performed to determine potential interactions. If the p-value for the ratio is <0.1, we will stratify antibody levels by the effect modifier and examine the effect of supplementation within each group.

9. General notes on statistical methods

9.1. Software

STATA 13 will be used to perform all the statistical analyses. Additionally, Graphpad Prism 5 will be used for constructing graphs. Database including all the variables for the analyses was constructed in Microsoft Excel and converted to dat* format to be used on STATA

9.2. Preparing malaria antibody data for analyses

Maternal peripheral plasma samples were heat inactivated and diluted to a working concentration prior to analysis. Both enrolment and 36gw samples for the same participant were assayed in the same plate on the same day. Every sample including the negative (malaria unexposed and non-immune Melbournian plasma) and positive (pool of sera collected from malaria immune individuals) controls were performed in triplicates

The MFI of antibodies were determined by taking the average of the triplicates (fluorimetry for determining antibodies to merozoite antigens) or duplicates (flow cytometry assays for VSA). The MFI for each sample is adjusted for intra and inter-plate variability. Intra-plate variability was determined by calculating the percentage variance of the MFI of the replicates. The samples were re-run if the variance between the triplicates/duplicates was greater than 20%, with their respective enrolment/ 36gw samples in the same assay.

Inter-plate variability was determined by calculating the percentage coefficient of variation (CV%) for each assay as following. Standard deviation of the MFIs of the positive control standard (highest dilution of the positive, see below) will be averaged for all the assays for each antigen and will be divided by the average MFI of the same positive standard for these assays to determine the CV as shown below.

$$CV\% = \frac{\text{Standard deviation}}{\text{Average}} \times 100\%$$

If the CV% for an assay was >30% this assay was repeated.

The positive pool sera was serially diluted to create a standard curve which was then used to determine the antibody levels as a percentage of the positive control with the lowest dilution set to 0% and the highest dilution set to 100%. Seropositivity for each antigen for each participant was determined using the formulas in section 5.4 and included in the database alongside the adjusted antibody levels.

9.3. Multiple comparisons

Bonferroni or Holm-Šídák method will be used for multiple comparisons.

Statistical adjustment for multiple comparisons for malaria immunity at 6 months will be performed for all the analyses as mentioned above; sections 8.5 – 8.6.

Similar adjustment for multiple comparisons for malaria immunity at 18 months will be performed for all the analyses as mentioned above; sections 8.7 – 8.8.

9.4. Confidence intervals

All the statistical analyses will be complemented with 95% confidence interval (CI) calculated based on t-test

9.5. Interactions and effect modifiers

9.5.1. We will test for interactions between the intervention groups and selected effect modifiers (list below) on their association with malaria antibody levels at enrolment and 36gw, magnitude and rate of antibody level change. All tests will be done using the likelihood ratio test.

1. Maternal age
2. Gravidity
3. HIV status
4. Bed net use
5. Season at enrolment
6. Malaria infection at enrolment(based on LM+ and LM-)
7. Neighborhood of residence (categorized based on the closest health centre)

Data will be obtained from Form 02, Q2.3; Q2.5; Form 06, Q3.3, Q6.2, Form 18, Q2.3, Form 03, Q2

9.5.2. Potential effect modifiers will be tested for any interactions between the intervention group and antibody levels and seropositivity at 6 and 18 months using likelihood-ratio test.

These variables include (as continuous variables where possible):

1. Maternal BMI at enrolment
2. Duration of gestation (from enrolment to delivery)
3. Number of pregnancies
4. Sex of the child
5. Maternal education
6. Proxy for SES
7. Study site
8. Maternal anaemic status at enrolment
9. Maternal HIV status
10. Bed net use by children

If a statistically significant interaction ($p<0.1$) is found, the outcome analysis will be completed as separate analyses for each stratum by the respective predictor variable.

9.6. Adjustment for covariates

Following covariates will be used to construct adjusted regression models for the outcome variables (antibody levels at 36gw, seroprevalence, magnitude of change and rate of change in antibody levels). If a statistically significant association was found (a $p<0.05$ level), these covariates will be included in all the four models – i.e. all the models will be adjusted for the same set of covariates.

1. No covariate adjustment
2. Maternal malaria antibody levels at enrolment
3. Malaria infection at enrolment
4. Maternal age
5. Gravidity

6. HIV status
7. Bed net use
8. Season at enrolment

9.6.1. Covariate adjustment for malaria immunity at 6 months.

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the outcome variables; antibody levels at 6 months and seropositivity. The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association (at $p<0.1$ level), will be included in the regression model

1. No covariate adjustment
2. Maternal BMI at enrolment
3. Duration of gestation (from enrolment to delivery)
4. Number of pregnancies
5. Sex of the child
6. Maternal education
7. Proxy for SES
8. Study site
9. Maternal anaemic status at enrolment
10. Maternal HIV status
11. Bed net use by children

9.6.2. Covariate adjustment for malaria immunity at 18 months.

The main analysis is planned to be completed and shown in tables and figures without any covariate adjustment.

As a secondary analysis, we will construct and show an adjusted regression model for the outcome variables; antibody levels at 18 months and seropositivity. The covariates to be included in the models will be derived from the list below. All variables which show a statistically significant association (at $p<0.1$ level), will be included in the regression model

1. No covariate adjustment
2. Maternal BMI at enrolment
3. Duration of gestation (from enrolment to delivery)
4. Number of pregnancies
5. Sex of the child
6. Maternal education
7. Proxy for SES
8. Study site
9. Maternal anaemic status at enrolment
10. Maternal HIV status
11. Bed net use by children

1. Tables

Table 1: Patient demographic and clinical characteristics

Characteristics	IFA	MMN	LNS	All women	P (95% CI)
No. pregnant women (maternal samples received at Melbourne)	XXX	XXX	XXX	1008	X.XX (XX to XX)
Gestation weeks (gw) at enrolment: median (IQR)	XX	XX	XX	17 (15-18.4)	X.XX (XX to XX)
Maternal age : median (IQR) <20 years No. (%) 20-25 26-30 >30	XX	XX	XX	24 (20-28) 310 (30.83%) 298 (29.62%) 238 (23.66%) 160 (15.90%)	X.XX (XX to XX)
Gravidity: Number (%) Primigravidae Secundigravidae Multigravidae (3-5 pregnancies) Grand multigravidae (>5 pregnancies)	XX	XX	XX	199 (19.76%) 202 (20.06%) 381 (37.84%) 225 (22.34%)	X.XX (XX to XX)
Malaria prevalence: Number (%) at enrolment, PCR Blood film	XX	XX	XX	XX (XX)	X.XX (XX to XX)
HIV prevalence: No: (%)	XX	XX	XX	130 (13%)	X.XX (XX to XX)
Anaemia: total (%) Haemoglobin levels Iron deficiency anaemia	XX	XX	XX	XX (XX)	X.XX (XX,XX)
Bednet use	XX	XX	XX	XX	X.XX (XX,XX)

Table 2: Seroprevalence to malaria among pregnant mothers across the supplementation groups at 36gw

	Pregnant women seropositive at 36gw/ total pregnant women in each group				**Comparison between LNS and MMN group**		**Comparison between LNS and IFA group**		**Comparison between MMN and IFA group**	
Variable	**LNS**	**MMN**	**IFA**	**P-value**	**Odds ratio (95 % CI)**	**P-value**	**Odds ratio (95 % CI)**	**P-value**	**Odds ratio (95 % CI)**	**P-value**
Total IgG to pregnancy- specific VSA	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Opsonising antibodies to pregnancy-specific VSA	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
VAR2CSA-DBL5	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Schizont extract	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Total IgG to non-pregnancy-specific VSA	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Opsonising antibodies to pregnancy-specific VSA	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
MSP-1 19kD	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx

MSP-2	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
MSP-3	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
EBA-175	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Rh2A9	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	xxx/xxx (xx.x %)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx

Data presented as the proportion of women seropositive at 36 gw (seroprevalence) for the 3 supplementation arms. Logistic regression will be performed adjusting for covariates and confounders

Table 3: Magnitude of antibody level change or rate of antibody level change categorized by supplementation groups

	Magnitude of antibody level change by 36gw or rate of antibody level change				**Comparison between LNS and MMN group**		**Comparison between LNS and IFA group**		**Comparison between MMN and IFA group**	
Variable	**LNS**	**MMN**	**IFA**	**P-value KW**	**Median difference (95 % CI)**	**P-value MW**	**Median difference (95 % CI)**	**P-value MW**	**Median difference (95 % CI)**	**P-value MW**
Total IgG to pregnancy- specific VSA, median (IQR)	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Opsonising antibodies to pregnancy-specific VSA	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
VAR2CSA-DBL5	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Schizont extract	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Total IgG to non-pregnancy-specific VSA	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Opsonising antibodies to pregnancy-specific VSA	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
MSP-1 19kD	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx

MSP-2	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
MSP-3	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
EBA-175	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx
Rh2A9	xx (xx, xx)	xx (xx, xx)	xx (xx, xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx	x.xx (x.xx-x.xx)	x.xxx

Data presented as median magnitude of change in antibody levels or median rate of antibody level and interquartile range (IQR). Comparisons between the 3 groups will be performed by Kruskal Wallis test (KW) while comparisons between 2 groups will be performed by Mann Whitney test (MW). Adjusted for covariates and confounders.

Table 4. Malaria antibody levels at 6 months stratified by supplementation groups

	Result by study group				**Comparison between LNS and IFA group**		**Comparison between LNS and MMN group**		**Comparison between MMN and IFA group**	
Outcome	**IFA**	**MMN**	**LNS**	**P-value[a]**	**Coefficient (95 % CI)**	**P-value[b]**	**Coefficient (95 % CI)**	**P-value[b]**	**Coefficient (95 % CI)**	**P-value[b]**
Number of participants	N=XXX	N=XXX	N=XXX							
MSP-1 19kD	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-2	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-3	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
EBA-175	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Rh2A9	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Schizont extract	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

Antibodies to VSA of E8B parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of R29 parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of 3D7 parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

a. P-value calculated using Kruskal Wallis test
b. Linear regression of antibody levels between supplementation groups
c. Multivariate regression adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

Table 5. Antibody seropositivity at 6 months by supplementation groups

	Number of children seropositive/ total number of children				**Comparison between LNS and IFA group**		**Comparison between LNS and MMN group**		**Comparison between MMN and IFA group**	
Outcome	**IFA**	**MMN**	**LNS**	**P-value[a]**	**RR (95 % CI)**	**P-value[b]**	**RR (95 % CI)**	**P-value[b]**	**RR (95 % CI)**	**P-value[b]**
MSP-1 19kD	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-2	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-3	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
EBA-175	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Rh2A9	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Schizont extract	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of E8B parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

Antibodies to VSA of R29 parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of 3D7 parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

a. P-value calculated using the Chi2 test
b. P-value calculated using logistic regression reporting Relative Risk Ratios (RR)
c. P-value calculated using multivariate logistic regression reporting odds ratios (OR) while adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

Table 6: Effect modification- dichotomous/continuous variables (by supplementation group)

		Result by study group				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
Outcome	Interaction test p-value[a]	IFA	MMN	LNS	P-value[b]	RR/difference in means (95 % CI)[c]	P-value[c]	RR/difference in means (95 % CI)[c]	P-value[c]	RR/difference in means (95 % CI)[c]	P-value[c]
Antibodies to MSP-1 19kD											
HIV=1	0.xx*	xxx/n (xx.x %)[1]	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)[2]	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
HIV=0		xxx/n (xx.x %)	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Primi = 0	0.xx*	xxx/n (xx.x %)[1]	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)[2]	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Multi = 1		xxx/n (xx.x %)	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Duration of gestation	0.xx*	mean (SD)	mean (SD)	mean (SD)	0.xxx	diff in means (x-y)	0.xxx	diff in means (x-y)	0.xxx	diff in means (x-y)	0.xxx

a. P-value calculated using likelihood ratio test
b. P-value calculated using Chi^2 test (dichotomous) or ANOVA (continuous, comparing mean differences)
c. Relative risk with 95 % confidence interval and the p-value

Table 7. Malaria antibody levels at 18 months stratified by supplementation groups

	Result by study group				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
Outcome	**IFA**	**MMN**	**LNS**	**P-value[a]**	**Coefficient (95 % CI)**	**P-value[b]**	**Coefficient (95 % CI)**	**P-value[b]**	**Coefficient (95 % CI)**	**P-value[b]**
Number of participants	N=XXX	N=XXX	N=XXX							
MSP-1 19kD	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-2	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
EBA-175	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Rh2A9	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Schizont extract	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of E8B parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

Antibodies to VSA of R29 parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of 3D7 parasite line	Median (IQR)	Median (IQR)	Median (IQR)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

a. P-value calculated using Kruskal Wallis test
b. Linear regression of antibody levels between supplementation groups
c. Multivariate regression adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

Table 8. Antibody seropositivity at 18 months by supplementation groups

	Number of children seropositive/ total number of children				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
Outcome	**IFA**	**MMN**	**LNS**	**P-value[a]**	**RR (95 % CI)**	**P-value[b]**	**RR (95 % CI)**	**P-value[b]**	**RR (95 % CI)**	**P-value[b]**
MSP-1 19kD	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
MSP-2	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
EBA-175	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Rh2A9	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Schizont extract	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of E8B parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Antibodies to VSA of R29 parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

Antibodies to VSA of 3D7 parasite line	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	xxx/xxx (xx.x%)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx
Adjusted model[c]					x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx	x.xx (xx.x, xx.x)	x.xx

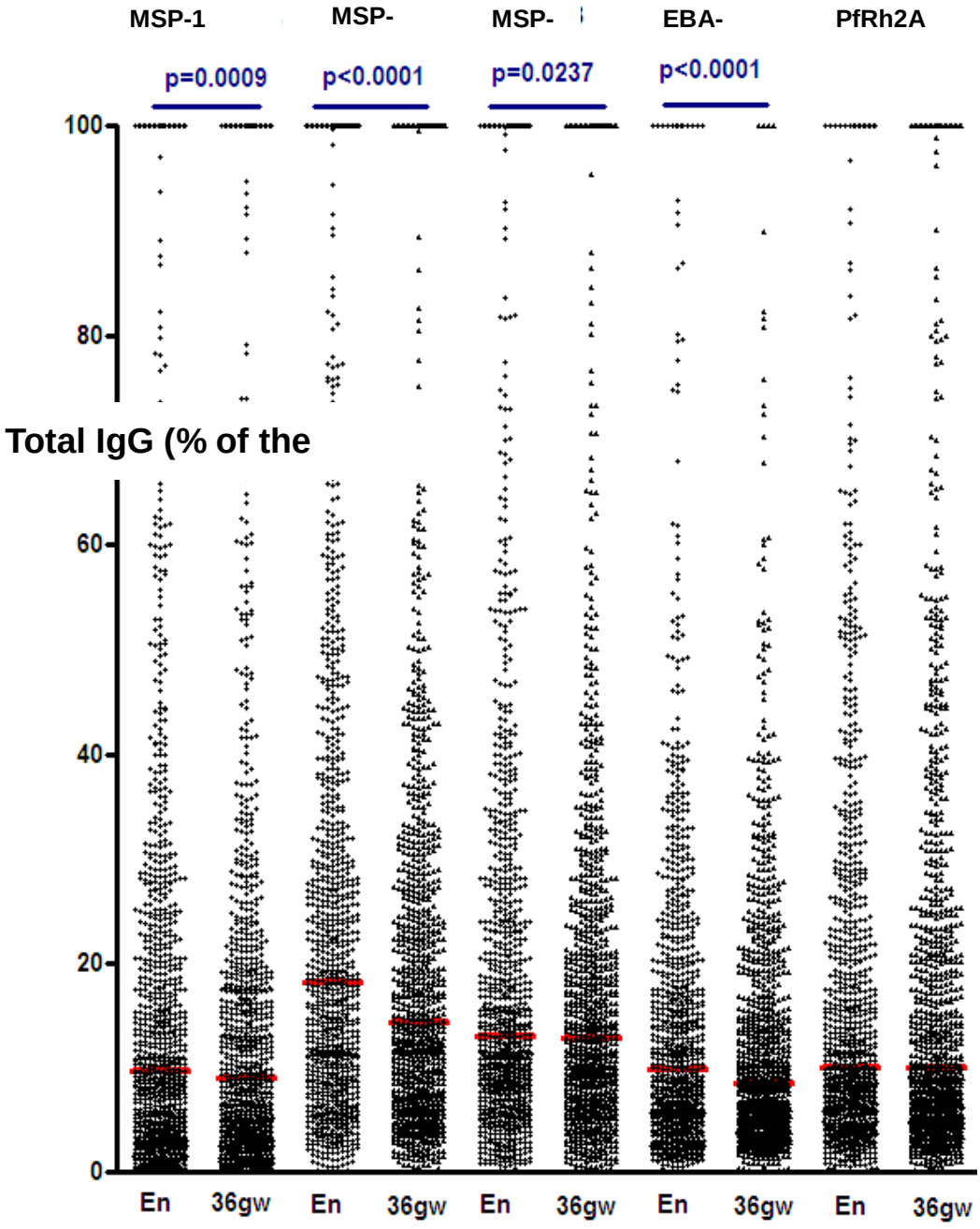
a. P-value calculated using the Chi2 test
b. P-value calculated using logistic regression reporting Relative Risk Ratios (RR)
c. P-value calculated using multivariate logistic regression reporting odds ratios (OR) while adjusting for sex of the child, maternal malaria, maternal HIV, maternal anaemia at 36 weeks, maternal education, bed net use, duration of gestation, socioeconomic status and study site

Table 9: Effect modification at 18 months- dichotomous/continuous variables (by supplementation group)

		Result by study group				Comparison between LNS and IFA group		Comparison between LNS and MMN group		Comparison between MMN and IFA group	
Outcome	Interaction test p-value[a]	IFA	MMN	LNS	P-value[b]	RR/difference in means (95 % CI)[c]	P-value[c]	RR/difference in means (95 % CI)[c]	P-value[c]	RR/difference in means (95 % CI)[c]	P-value[c]
Antibodies to MSP-1 19kD											
HIV=1	0.xx*	xxx/n (xx.x %)[1]	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)[2]	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
HIV=0		xxx/n (xx.x %)	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Primi = 0	0.xx*	xxx/n (xx.x %)[1]	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)[2]	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Multi = 1		xxx/n (xx.x %)	xxx/n (xx.x %)	xxx/n (xx.x %)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx	RR (x-y)	0.xxx
Duration of gestation	0.xx*	mean (SD)	mean (SD)	mean (SD)	0.xxx	diff in means (x-y)	0.xxx	diff in means (x-y)	0.xxx	diff in means (x-y)	0.xxx

d. P-value calculated using likelihood ratio test
e. P-value calculated using Chi^2 test (dichotomous) or ANOVA (continuous, comparing mean differences)

2. Figures and legends



Example figure 1: Antibody levels to merozoite antigens reported as a percentage of the positive control at enrolment (En) and 36 gestation weeks (36gw). Each symbol represents an individual pregnant woman with %total IgG. Red horizontal bar indicates the median and the yellow dashed line denotes the average seronegative cut off. Sample size, n=1008.

Figure 2: Bar graph representing magnitude of antibody level change categorised by supplementation groups.

Figure 3 A: Antibodies to merozoite antigens and schizont extract at 6 months by supplementation group

Figure 3 B: Antibodies to variant surface antigens at 6 months by supplementation group

Figure 4 A: Antibodies to merozoite antigens and schizont extract at 18 months by supplementation group

Figure 4 B: Antibodies to variant surface antigens at 18 months by supplementation group