Analysis of measles Immunoglobulin-G (IgG) concentration of children at 6 and 18 months of age

Statistical Analysis Plan (Addendum to "Main" SAP, version 3)

Contents

Approval

Version number	1
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Version History Log

This table will detail the version history for this document, including the key elements of the changes to the versions.

Version	**Date implemented**	**Details of significant changes**
1	2015 Feb 18	This is the first version of this SAP. It is intended as an addendum to the main SAP, Version 3. As a result, there are various aspects of the analysis that have been described in the main SAP Version 3 and will not be repeated here. Both the main SAP Version 3 and this current SAP (*Measles IgG concentration of children at 6 and 18 months of age*) will guide the analysis described in this document.

1. Background

This analysis will involve a sub-sample of infants who were born to women who participated in the iLiNS DYAD Study in Ghana (ref). In this study, pregnant women (n =1320, ≤20 weeks gestation) were randomized to receive either standard iron (60 mg) + folic acid (400 μg) during pregnancy and placebo (small amount of Ca) in the first six months postpartum (IFA group), or 18 micronutrients including 20 mg iron (MMN group), or small-quantity Lipid-based Nutrient Supplement (LNS group) during pregnant and first six months postpartum. The LNS group received the same micronutrients as the MMN group, and in addition Ca, P, K and Mg as well as macronutrients. Infants of mothers in the IFA and MMN groups received no supplementation, but those of mothers in the LNS group received small-quantity Lipid-based Nutrient Supplement (SQ-LNS) designed for infants from 6 to 18 months age.

During follow-up from birth to 18 months of age, infants attended routine "well-baby" clinics monthly, and received vaccines and high dose Vitamin A in accordance with Ghana Health Service (GHS) guidelines. Measles vaccine was received at the clinic visit that was closest to nine months of age. In February 2012, just over three years into the study, the Ministry of Health introduced the policy of a second measles vaccine dose to be received between 13 and 18 months of age. As a result, 55 of the infants in the sub-sample were documented to have received a second dose of the measles vaccine.

2. Procedures

Blood samples of infants were collected at 6 and 18 months of age during scheduled laboratory visits, and were subsequently shipped on dry to UC Davis for analysis. In a sub-sample of infants (n = 475) for whom adequate plasma samples were available for 6 and 18 months of age, we measured plasma measles Immunoglobulin G (IgG) antibody using a commercially available ELISA kit. The assay included internal standards for the quantitative evaluation of measles IgG concentrations that have been adjusted to a WHO international standard (3rd International Standard for Anti-Measles, National Institute for Biological Standards and Control [NIBSC] code 97/648). All samples were plated in duplicate. The results of the assay are expressed as mIU/ml.

3. Study objectives

To determine the effect of intervention treatment on the measles IgG concentration of the iLiNS DYAD infants.

Specific objectives are to:

1) Compare mean measles IgG concentration (mIU/mL) of infants of the three intervention groups at 6 months of age (before the eligible age for measles vaccination) and at 18 months of age (following measles vaccination), as well as the change in concentration between 6 and 18 months or ratio of concentration at 18 months to concentration at 6 months

2) Compare measles-seropositive rate at18 months of age.
3) Evaluate whether the effects of the intervention on measles IgG concentration (mIU/mL), change in measles IgG concentration or ratio of IgG concentration, and measles-seropositive rate at 18 months are modified by pre-specified maternal and infant factors.

4. Hypotheses

a) Infants in the LNS group (whose mothers received LNS during pregnancy and lactation, and who themselves received LNS for infants from 6 to 18 months of age) will have greater mean measles IgG concentrations (mIU/mL) at 18 months of age, and a greater change from 6 to 18 months or ratio of concentration at 18 months to concentration at 6 months, than infants in the other two groups.
b) Measles seropositivity rate will be greater in the infants in the LNS group than those in the other two groups.

5. Outcome measures

Outcome measures are:

a) Measles IgG concentration (mIU/mL) at 18 months of age.
b) Change in measles IgG concentration between 6 and 18 months of age, or ratio of measles IgG concentration at 18 months to measles IgG concentration at 6 months.
c) Percentage of infants who are seropositive at 18 months of age following measles vaccination.

6. Definition

Seropositivity at 18 months of age will be defined as measles IgG concentration ≥200 mIU/mL, based on data suggesting that measles IgG concentrations >200 mIU/ml protect against natural infection (1, 2).

7. Basis for the analysis

All children in the sub-sample (for whom plasma samples were available at both 6 and 18 months) will be analyzed regardless of the percentage of follow-up days in which the mother reportedly consumed her own supplement, or gave LNS to the child.
A sensitivity analysis will be performed which will be restricted to infants who were documented to have received the measles vaccine dose at 9 months only, and not a second vaccine dose.

8. Statistical software

Analyses will be performed using SAS version 9.3 (SAS Inst., Cary, NC, USA).

9. Outliers

Outliers will be visually inspected by creating box and whisker plots and/or histograms of individual continuous variables, and scatterplots of related variables. Outliers which are clearly

impossible or implausible values will be corrected if possible, or recoded to missing if correction is not possible. Outliers which are plausible or possible will be kept.

10. Data transformation

Distribution of continuous outcome variables will be inspected for normality and transformed as necessary. If no suitable transformation is found, normalized ranks will be calculated, or categories will be created.

11. Analytical approach

a. To assess whether the sub-sample of infants included in this analysis adequately represented the entire sample, the background characteristics of the sub-sample versus those of infants not included in this analysis will be compared using student's t-test (continuous variables) and chi-squared test (categorical variables). These results will be presented as means ±SD (continuous variables) or frequencies (binary variables) along with p-values (**Table 1**).

b. Outcome measures among treatment groups will be evaluated using general linear model (continuous outcomes) and logistic regression model (binary), with Tukey-Kramer adjustment for multiple comparisons. Along with the treatment group comparisons, we will calculate pairwise mean differences (continuous outcomes) and relative risks (binary outcomes) with their 95% CI and p-values. Relative risks will be calculated using Poisson regression (3). We will analyze each outcome twice, first without any covariate adjustments, and then with adjustment for covariates (see below) significantly associated ($p<0.10$) with the outcome in a bivariate analysis.

c. For those continuous outcome variables that are not normally distributed, the group means (± SD or SE), group percentages, and pair-wise mean differences and relative risks with their 95% CI will be calculated using untransformed data to allow for easy interpretation of results, while the p-values for group or pair-wise comparisons will be generated using logarithmically transformed data. Results of the analysis of continuous and binary outcomes will be presented as shown in **Table 2** and **Table 3**, respectively.

d. For each outcome, we will evaluate potential effect modification by pre-specified background variables (see below). Where an effect modification is significant (alpha <0.10), we will perform stratified/subgroup analysis by including an interaction term between treatment and the effect modifier in the ANCOVA or logistic regression model. Each effect modifier will be considered separately in the models to avoid collinearity. Results of sub-group analyses will be presented as shown in **Table 4**.

12. Potential covariates

The covariates to be included in the ANCOVA or logistic regression models will be derived from the list below. Each variable that shows a statistically significant association with an outcome ($P<0.10$) will be included in the model.

a. Maternal variables at enrolment
   1. BMI (continuous)
   2. Inflammatory markers (CRP and/or AGP: binary)
   3. Primiparity (binary)
   4. Season at enrolment (binary)
   5. Anemia (binary)
   6. TfR at enrollment (continuous)
   7. Gestational age (continuous)
   8. Age (continuous)
   9. Assets index (continuous)
   10. Housing index (continuous)
   11. HH food insecurity index (continuous)

b. Infants' variables
   1. Sex (binary)
   2. Number of days from most recent vaccination to sample collection (continuous)
   3. Vitamin A doses received (binary: 3, or less than 3)
   4. Measles vaccine doses received (1, or more than 1)
   5. Measles IgG concentration at 6 months

13. Potential effect modifiers

Potential effect modifiers to be examined for measles IgG concentration (mIU/mL) and measles-seropositive rate at 18 months of age are listed below. For significant effect modifiers, sub-group analyses will be based on $<10^{th}$ percentile value versus $< 90^{th}$ percentile for continuous effect modifiers, and "yes or no" for binary effect modifiers.

a. Maternal variables at enrolment
   1. BMI (continuous)
   2. Inflammatory markers (CRP and/or AGP: binary)
   3. Primiparity (binary)
   4. Season (binary)
   5. Anemia (binary)
   6. TfR at enrollment (continuous)
   7. Gestational age (continuous)
   8. Age (continuous)
   9. Assets index (continuous)
   10. Housing index (continuous)
   11. HH food insecurity index (continuous)

b. Infants' variables
   1. Sex=male (binary)
   2. Number of days from most recent vaccination to sample collection (continuous)
   3. Vitamin A doses received (binary: 3, or less than 3)
   4. Measles vaccine doses received (1, or more than 1)
   5. Measles IgG concentration at 6 months

Reference

1. Bautista-Lopez NL, Vaisberg A, Kanashiro R, Hernandez H, Ward BJ. Immune response to measles vaccine in Peruvian children. Bulletin of the World Health Organization 2001;79(11):1038-46.
2. Markowitz LE, Preblud SR, Fine PE, Orenstein WA. Duration of live measles vaccine-induced immunity. The Pediatric infectious disease journal 1990;9(2):101-10.
3. Spiegelman D, Hertzmark E. Easy SAS calculations for risk or prevalence ratios and differences. Am J Epidemiol 2005;162(3):199-200. doi: 10.1093/aje/kwi188.

Table 1
Background characteristics of children included in the analysis and those not included in the analysis

Variable	**Included ($\bar{x}$ ± SD) [n]**	**Excluded ($\bar{x}$ ± SD) [n]**	**P**
Number of infants	xxx	xxx	
Maternal variables at enrolment			
Age, y	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Years of formal education, y	xx.x ± x.x [xx]	xx.x ± x.x [xx]	x.xxx
Married or cohabiting, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Proxy for household SES	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xxx
Primiparity, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Weight, kg	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Height, m	x.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xxx
BMI, kg/m^2	xx.x ± x.x [xxx]	x.x ± x.x [xxx]	x.xxx
Low BMI (< 18.5 kg/m²), n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Mid upper arm circumf, cm	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Triceps skinfold, cm	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Gestational age , weeks	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Positive rapid test for malaria, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Infants' variables			
Sex = meals, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Gestational age delivery, weeks	xx.x ± x.x [xxx]	xx.x ± x.x [xxx]	x.xxx
Received one Vitamin A dose, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Received two Vitamin A doses, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Received three Vitamin A doses, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Received one measles vaccine dose, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx
Received two measles vaccine doses, n (%)	xx/xxx (xx.x%)	xx/xxx (xx.x%)	x.xxx

Table 2
Unadjusted and adjusted mean measles IgG concentrations and pairwise-differences (mIU/mL) at 6 and 18 months of age

	IFA [n]	MMN [n]	LNS [n]	P-value	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Difference in means or medians (95 % CI)	p	Difference in means or medians (95 % CI)	p	Difference in means or medians (95 % CI)	p
6 months of age										
Unadjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
Adjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
18 months of age										
Unadjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
Adjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
Change from 6 to 18 months of age										
Unadjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
Adjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
18 months/ 6 months ratio										
Unadjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx
Adjusted	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [x]	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx	x.xx (x.x, x.x)	x.xxx

Table 3

Unadjusted and adjusted and measles seropositivity rate at 18 months of age

	IFA n (%)	MMN n (%)	LNS n (%)	P-value	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					RR (95 % CI)	P	RR (95 % CI)	P	RR (95 % CI)	P
Unadjusted	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx
Adjusted	x (x.x)	x (x.x)	x (x.x)	0.xx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	x.xx (x.xx, x.xx)	x.xxx

Table 4

Subgroup analysis of measles IgG concentration and measles seropositivity at 18 months of age[1]

Outcome variable	IFA[2]	MMN[2]	LNS[2]	P[3]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
					Mean difference or RR	p	Mean difference or RR	p	Mean difference or RR	p
IgG concentration										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Change from 6 to 18 months										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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

Outcome variable	IFA[2]	MMN[2]	LNS[2]	P[3]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
					Mean difference or RR	p	Mean difference or RR	p	Mean difference or RR	p
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
18 months/ 6 months ratio										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Seropositivity										
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Effect modifier				x.xxx		.		.		.
Sub-group #1	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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
Sub-group #2	x.x ± x.x [x]	x.x ± x.x [x]	x.x ± x.x [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

[1]IFA= Iron-Folic Acid group received 60 mg iron plus 400 µg folic acid; MMN=Multiple Micronutrients group received 1-2 RDAs of 18 vitamins and minerals (including 20 mg iron); LNS=Lipid-based nutrient supplement (LNS) group received LNS with same micronutrients as the MMN group, plus another four minerals (Ca, P, K and Mg) as well as macronutrients. All three supplements were intended for daily consumption.

[2]Data are means ± SE for continuous effect modifiers, or means ± SE [n] for binary effect modifiers.

[3]P-values with asterisks are for interaction. P-values without asterisks compare all three groups in each stratum.