LAMPREG study

Statistical Analysis Plan (SAP) statement

Version 1.0 – February 2021

Section 1: Administrative information

1. Title and Trial registration

Statistical analysis plan for the LAMP for Malaria in Pregnancy (LAMPREG) trial: "Active case detection and treatment of malaria in pregnancy using LAMP technology: A pragmatic randomized multi-Center diagnostic outcomes trial"

This trial is registered in clinicaltrial.gov: registration number NCT03754322

2. SAP version

Version: 1.0, Date: February 22nd, 2021

3. Protocol version

This document has been written based on information contained in the study protocol version 1.5, dated February 22nd, 2021.

4. SAP revisions – revision history

Protocol version	Updated SAP version no.	Section number changed	Description of and reason for change	Date changed
1.5	1.0	First SAP version		February 22nd, 2021

5. Roles and Responsibility – non signatory names and contribution

This SAP protocol was written and designed by

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6. Roles and Responsibility – signatures

- Dr Dylan R. Pillai, MD, PhD, PI of the LAMPREG study

Name	Name
Signature: _____	Signature: _____
Date: _____	Date: _____

Section 2: Introduction

1. Background and rationale

Malaria is an infectious disease transmitted by *Anopheles* mosquito. Pregnant women are particularly susceptible to adverse outcomes in terms of maternal and fetal health. Several prevention strategies are available to public health authorities to prevent malaria. These strategies are anti vectorial (bed nets, monitoring of stagnant water where mosquito larva bread), intermittent preventive treatment or repetitive screening during antenatal care (ANC). All the above mention strategy present limitations that impair their efficiency to prevent malaria-associated morbidity.

The LAMPREG trial aims to fill the knowledge gap by proposing an alternative systematic screening strategy using point of care (POC) compatible molecular biology techniques.

2. Objectives

Research hypothesis: The trial hypothesis is that enhanced LAMP-based malaria detection during ANC will lower the proportion of malaria-associated adverse outcomes (low birth weight, pregnancy loss, anemia). The proportion of Low Birth weight is the study primary outcome.

Primary objective:

The primary objective of this trial is to evaluate the effectiveness of LAMP-based enhanced malaria detection in improving pregnancy outcomes as measured by birth weight, neonatal anemia, maternal anemia, and stillbirth.

Secondary objective:

The secondary objectives are:

a. To determine the proportion of asymptomatic MiP cases revealed by microscopy, RDT and LAMP
b. To evaluate the impact of the intervention in terms of malaria-associated placental damage as evaluated by placenta weight and placental malaria.
c. To evaluate the performances of IllucidX LAMP(ultrasensitive) compared to the approved method using the samples from the trial.
d. To compare the diagnostic performance of microscopy, RDT and LAMP against qRT-PCR in MiP.
e. To decipher parasitic genotypes that may impair diagnostic test performances and treatment efficacy using parasite whole-genome sequencing.

Section 3: Trial Methods

1. Trial design

The study is a prospective diagnostic study of malaria in pregnant women. The goal is to determine whether: (i) LAMP provides a clinically measurable benefit compared to current first-line diagnostic test of Giemsa-stained microscopy and whether (ii) enhanced case detection of asymptomatic mothers with LAMP has added value in terms of outcomes. We hypothesize that addition of LAMP to one arm will be of greater benefit than microscopy alone due to additional LAMP sensitivity. We further hypothesize that enhanced case detection by screening asymptomatic mothers at each antenatal visit will be of additional value in treating malaria. Both symptomatic and asymptomatic first and second trimester mothers will be included in the study and individually randomized to one of two arms: standard of care or enhanced cased detection arms using LAMP for malaria. Mothers will be enrolled during a twelve-months period from March 2021 to July 2022 then followed until 28 days after delivery

2. Randomization

Women will be screened for eligibility and randomized either in the Standard of Care (SOC, 1/3 of included women) arm or in the intervention arm (2/3 of included women).

Randomization will be implemented at each health center. Stratification per parity (primiparus, second pregnancy and ≥ 3 pregnancy) will be implemented to avoid confounding factor of acquired immunity for MiP variants.

3. Sample size

The sample size calculation for this study is based on detecting a difference in the proportion of deliveries with a low birth weight (LBW), which was felt to be the most clinically important outcome variable. The prevalence of deliveries with a LBW in Ethiopia was estimated to be 17.3%. An alpha of 0.05 was selected with an allocation ratio of 3:1 in favor of the enhanced case detection arm. The study will be powered to detect an absolute difference in the proportion of deliveries with a LBW of 5%, so a proportion in the enhanced case detection arm of 12.3% compared to 17.3% in the standard of care arm. Using a continuity correction and an attrition rate of 20%, a total sample size of 2583 is required to achieve 80% power.

4. Framework

The LAMPREG trial aims to show the superiority of the intervention arm.

5. Statistical Interim analyses and stopping guidance

a. Two formal interim analysis are planned

- One formal statistical interim analysis is planned when 25% of the total inclusion target number (n = 646) is reached. This interim analysis will focus on the inclusion baseline data and the follow-up data available to date.
- Another formal statistical interim analysis is planed when 25% of the inclusion target reached the end of the protocol (28 days post-natal delivery). This analysis will be partial since post hoc gold standard molecular testing will not be available yet.

b. Adjustments planned of the significance level due to interim analysis

The prevalence of malaria in the study sites is paramount for the study outcome. Interim analysis will tailor this factor and decision of site closure or site initiation may be taken.

c. Details of guidelines for stopping the trial early

The LAMPREG trial interim results will be presented to an advisory board. LAMPREG is not a new interventional drug trial, and therefore unlikely to be subject to study termination. The Ethiopian NRERC ethical board waived the need of independent data monitoring and safety committee for LAMPREG.

6. Timing of final analysis

The final analysis of clinical data in the SOC *vs.* LAMP arm will be performed after the last follow up is completed, in a time frame of 6 months (secund semester of 2022), given that data entry is performed according to the protocol.

The laboratory testing post trial (gold standard testing, parasitic variant assessment) is scheduled for 2 years post sample collection, with analysis and publication of results within a 3-year frame (end of 2022- first semester of 2023).

7. Timing of outcome assessments

The scheduled of ANC visit is given in the study protocol (v1.5 – see table 5).

Section 4: Statistical Principles

1. Confidence intervals and p-values

All applicable statistical tests will be two-sided and will be performed using a 5% significance level.

All confidence intervals presented will be 95% and two-sided.

2. Adherence and Protocol Deviations

Protocol adherence is defined by the completion of all ANC visit as per study arm (included treatment course completion for women eligible) and the follow-up of the newborn for 28 days post-delivery.

Nonadherence is defined by the number of missed visits, the loss to follow-up or the absence of recorded birth weight.

Protocol deviation is defined as:

- Non-administration of malaria treatment for LAMP positive women in the intervention arm
- Incomplete treatment administration for any treated women
- Loss to follow up or partial course of ANC visit

3. Analysis population

The intention-to-treat population will include all randomized patients, according to the study arm they were randomized in.

Section 5: Trial population

1. Screening data

The following summaries will be presented for all screened patients: inclusion time frame, number of the screened expecting mothers, number of mothers recruited per month, the reason for non-recruitment. This summary will be presented overall and by study center.

2. Eligibility

Eligibility criteria are presented in the study protocol v1.5.

To summarize, all pregnant women >18 years old, with gestational age < 27 weeks and attending ANC care in any of the study centers is eligible to the study

3. Recruitment

The "CONSORT" diagram comprising the number of people screened, eligible, consented, randomized, receiving their allocated intervention (malaria diagnostic and treatment) and followed to delivery will be presented with the study results.

4. Withdrawal/Follow-up – level of withdrawal

The level of consent withdrawal will be tabulated (classified as "consent to continue follow-up, sample and data collection" "consent to continue follow up and data collection ", "complete –no further follow-up or

data collection", "lost to follow up". This will be presented in CONSORT diagram format rather than as a table, with numbers and reasons for withdrawal and/or exclusion from the analysis given at each stage. The numbers (with reasons) of losses to follow-up (dropouts and withdrawals) over the course of the trial will be summarized by treatment arm.

5. Population and subgroup to be analyzed

Intention-to-treat (ITT): All randomized study subjects. This will be the primary population for the analysis.

Per Protocol (PP): All randomized study subjects completing the whole study period (complete cases). For a specific analysis, study subjects with missing data on any of the variables in the model will be excluded from the analysis. Analyses of this population is seen as a sensitivity analysis to investigate whether conclusions are sensitive to assumptions regarding the pattern of missing data

We will analyze independently the following subgroups (Both ITT and PP populations):

a. Primiparous mother
b. **Asymptomatic malaria**: women who did not present symptom at any ANC visit but were detected positive for *Plasmodium* spp infection by any diagnostic method.
c. **Sub microscopic malaria**: any women diagnosed positive for malaria by molecular method (LAMP or post hoc gold standard RT-qPCR)
d. **Malaria species identified**: *P. falciparum, P. vivax* and mixed infections (3 subgroups)
e. Women presenting positive malaria testing in placental blood at delivery
f. Women from newborn with Low Birth Weight

6. Baseline patient characteristics

Expectant women will be described with respect to age, parity, gestity, gestational age, history of malaria, pregnancy complication, both overall and separately for the two randomized groups. These data will be summarized in a table.

Section 6: Analysis

1. Outcome definition

Primary study outcomes are related to newborn health: fetal anemia (hemoglobin <12·5 g/dL), mean birthweight, low birthweight (<2500 g), mean gestational age, preterm delivery (<37 weeks' gestation), mean birthweight for gestational age (Z scores), small for gestational age, fetal loss (spontaneous abortion at <28 weeks' gestation or stillbirth), and the composite outcomes of adverse livebirth (preterm, low

birthweight, or small for gestational age) and adverse pregnancy (adverse livebirth or fetal loss). Mortality outcomes included neonatal, perinatal, and mortality up to age 4 weeks.

Secondary outcomes were signs of placental malaria infection at delivery, defined as a composite of maternal malaria (detection of infection in peripheral blood with post hoc gold standard testing) or placental malaria (detection of infection in placental blood with microscopy, molecular testing for active infection, or malaria pigment in blood smear monocytes for signs of past infection)

Secondary outcomes include maternal anemia (any: hemoglobin level <11 g/dL; moderate: hemoglobin level <9 g/dL) during each ANC visit and at delivery. We will use the categories "no anemia", "mild anemia", "moderate anemia" and "severe anemia" using the reference values for each pregnancy trimester. Values will be disaggregated according to gestational age.

Secondary outcomes during pregnancy comprised maternal malaria, detected with any method and by each method separately. This outcome was further stratified by patent infection (positive microscopy or malaria rapid diagnostic test) and sub-patent infection (negative microscopy and malaria rapid diagnostic test and positive LAMP–PCR). Morbidity outcomes assessed during pregnancy comprised clinical malaria (documented or history of fever plus positive malaria rapid diagnostic test or microscopy) and unscheduled clinic visits for any reason and for all reasons unrelated to malaria.

2. Analysis methods

Mean values of quantitative variables will be compared using a two-sided t-test with a significance of $p < 0.05$. We will use a negative binomial model to assess the effect of asymptomatic malaria screening using the number of (i) both microscopic and submicroscopic and (ii) submicroscopic *P. falciparum* infections occurring during pregnancy with an adjustment for the confounding variables. Multiple test correction will be implemented.

Relative risk (RR) and corresponding 95% confidence intervals (CIs) of adverse birth outcomes and adverse pregnancy based on malaria status will be calculated using log-binomial regression with a log link function. We will use a multivariable quantile regression to further assess the effect of malaria infection on birth weight and gestational age at delivery. Multivariable models will be adjusted for co factors. Adjusters will be included in multivariable analyses based on an a priori hypothesized relationship with the outcome of interest and will be further considered if they had a $p < 0.05$ in bivariate analysis. No variables chosen as adjusters by substantive knowledge will be excluded based on the results of bivariate analysis. Ultimately, all variables included in the models, including those identified by bivariate screening, will be chosen among variables documented as risk factors for adverse birth outcomes and related to MIP.

3. Missing data

We will use simple imputation for missing covariates (<1%). For outcome variates, we will no implement imputation.

For birth weight, correction factors will be implemented for those collected outside of the study framework.

4. Additional analyses

N/A

5. Harms

6. Statistical software

The data entry is performed using RedCap. Data are exported in STATA and CSV format for analysis in Stata and R.

7. References

Statement references