Radboudumc, Department of Medical Microbiology

# Safety, Tolerability and Plasmodium falciparum transmission-reducing activity of R0.6C vaccine adjuvanted with Alhydrogel alone or combined with Matrix-M in healthy malaria-naïve adults in the Netherlands (STOP-TRANS)

## Statistical analysis plan

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# Signature sheet

The signature below constitutes approval of this statistical manual.

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# List Of Abbreviations And Relevant Definitions

| | |
|---|---|
| AE | Adverse event |
| AUC 0-∞ | Area under the concentration versus time curve extrapolated to infinity |
| Cmax | Maximum observed serum concentration |
| CMV | Cytomegalovirus |
| CL | Drug clearance |
| ELISA | Enzyme-Linked Immuno Sorbent Assay |
| GMT | Geometric mean titers |
| HIV | Human immunodeficiency virus |
| mAB | Monoclonal Antibody |
| PD | Pharmacodynamics |
| PK | Pharmacokinetics |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SMC | Safety Monitoring Committee |
| SMFA | Standard Membrane Feeding Assay |
| SOP | Standard operating procedure |
| $T_{1/2}$ | Terminal half-life |
| TBA | Transmission blocking activity |
| TRA | Transmission reducing activity |
| $V_d$ | Volume of Distribution |

## Purpose

The purpose of the statistical analysis plan (SAP) is to outline the analyses that will be applied to the data of this trial. The SAP serves as a supplement to the protocol and contains further details about the study procedures to analyze the safety data, antibody data and standard membrane feeding assay (SMFA) data of participants of the trial.

# Introduction

R0.6C is a first-in-human phase I, open-label, single-site, dose escalation study to determine the safety, tolerability and transmission reducing activity of the R0.6C vaccine in two different adjuvant combinations (Alhydrogel alone and Alhydrogel combined with Matrix-M) and at two doses (30 µg and 100 µg). The objective of the study is to assess safety and tolerability of the R0.6C vaccine and measure transmission-reducing activity by standard membrane feeding assay (SMFA). R0.6C is a recombinant vaccine that induces an immune reaction against Pfs48/45, a protein expressed at the gametocyte stage of *Plasmodium falciparum*. An effective transmission-blocking vaccine shall be an important tool in controlling the spread of *P. falciparum.*

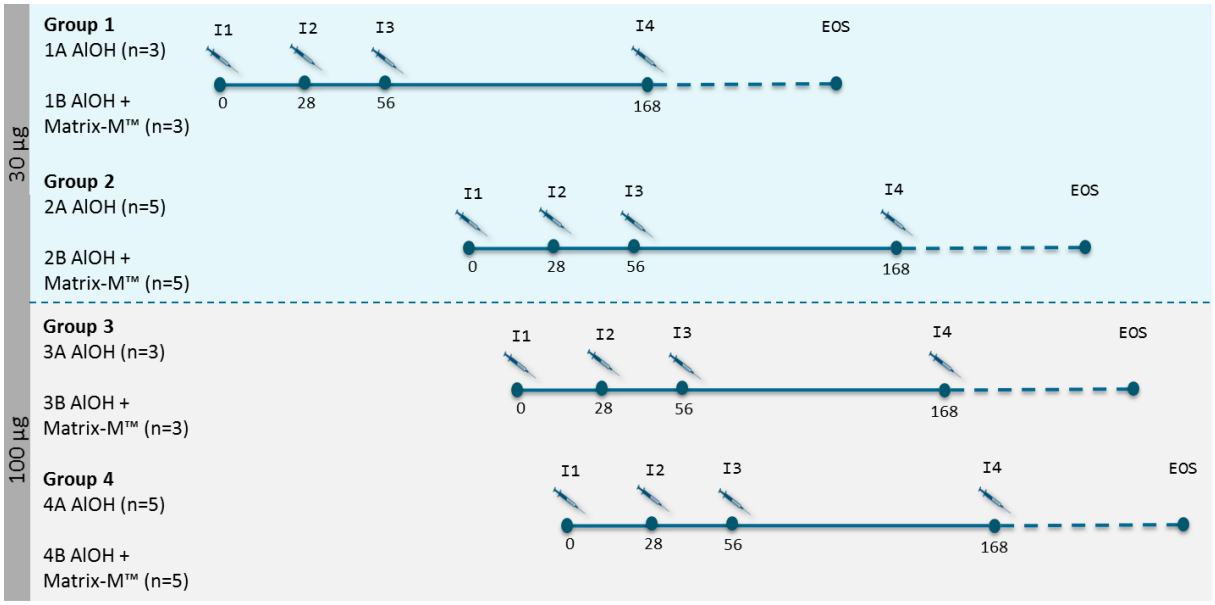


Figure 1 – Schematic study design

# Methods

## Study design

This is a first-in-human phase I, open-label, single-site trial. A total of 32 healthy, malaria naïve adults (males and females), aged 18 – 55 years will be included in the trial. Participants will receive four intramuscular vaccinations on days 0, 28, 56 and 168 with R0.6C adsorbed to Alhydrogel alone (Groups 1-4A), or combined with a second adjuvant: Matrix-M (groups 1-4B). Dose escalation with the two adjuvant arms will take place in parallel: first, a sentinel group (1A and 1B, n=3 per arm) will receive the low dose of 30µg R0.6C; subsequently the additional subjects (groups 2A and 2B, n=5 per arm) will receive the low dose of 30 µg R0.6C; if considered safe, a sentinel group (3A and 3B, n=3 per arm) will receive the high dose of 100µg R0.6C; and finally, the remainder of subjects will receive the high dose of 100µg R0.6C (groups 4A and 4B, n=5 per arm). The study participants are followed up to 3 months after the last vaccination; samples are collected following a scheme defined in the study protocol.

## Randomization

Randomization will be done for the allocation to one of the two different adjuvant combinations (arms A or B). The volunteers will be allocated to one of the two adjuvant arms per group at random using a Mersenne-Twister random number generator implemented in R through the command `sample()` and a pre-specified seed.

## Sample size

STOP-TRANS is an exploratory first-in-human study with the objective to identify large safety and tolerability signals. In addition, SMFAs are done to provide a first proof-of-concept. The study is largely descriptive and is intended to provide primarily safety data of 2 dose levels of R0.6C combined with 1 or 2 adjuvants given 4 times over 24 weeks. Comparative statistics will be performed but will have low power to detect anything other than very large differences between the groups. In total 32 participants will receive R0.6C. Based on a binomial distribution, this means that the probability to detect at least one clinically significant event (e.g. a serious adverse reaction) during vaccination with a power of 90% is 7%.

Furthermore, if we assume an effect size of 75% TRA at 14 days after the fourth R0.6C vaccination (I4+14) compared to baseline (I1-1), with the subject size of n=8 per dose/adjuvant arm we will have an empirical power of >99% (α=0.05) to detect this, based on calculations and assumptions as described in the next paragraph.

## Estimation of empirical power for an analysis of transmission reducing activity under different assumptions

### The simulation algorithm

1. We generate $n_0$ subjects in the control (pre-treatment) group and $n_1$ subjects in the treatment (post-treatment) group with a variable $x_i$ to indicate whether the subject is in the control group $(x_i = 0)$ or in the treatment group $(x_i = 1)$.
2. We generate $m$ mosquito dissections for each subject.
3. We simulate subject-specific random intercepts $(z_i)$ from a normal distribution with a mean of 0 and a specified standard deviation $\sigma$, i.e. $Z \sim N(0, \sigma)$.
4. Lastly we generate the oocyst counts $(y_{ij})$ for each subject $i$ and mosquito $j$ using a negative binomial distribution such that $Y \sim NB(\mu, \theta)$ where $\theta$ is the anticipated dispersion parameter and $\mu_i j = \exp(\beta_0 + \beta_1 x_{ij} + z_i)$ where $\beta_0$ is the anticipated mean of the log oocyte counts in the control group and $\beta_1$ is the anticipated regression coefficient used to estimate the TRA, such that

$$\beta_1 = \log\left(1 - \frac{TRA}{100}\right).$$

### The model

To analyze the simulated data, we make the of the $mgcv$ package in R. We run a Generalized Additive Mixed Model such that,

$$\log(y) = \beta_0 + \beta_1 x + z_i.$$

From each simulated dataset, we estimate the $TRA = \left(1 - \exp\left(\hat{\beta}_1\right)\right) \times 100$ and $p$ (the p-value) of the test for whether $\beta_1$ is significantly different from $0$. We then report the average $TRA$ across simulations, which should be close to the anticipated value, and the emperical power which we define as the percentage of $p$'s less than 0.05.

### Simulation results

We chose $m = 20$ as the standard amount of mosquito dissections per subject. We used a previous study (NL69779.091.19) to extract anticipated values for the mean log oocyst count in the control group, the dispersion parameter $\theta$ and the standard deviation of the random effects $\sigma$. i.e. $\beta_0 = 3.7037$, $\theta = 4.451$ and $\sigma = 0.64$. For the anticipated $TRA$ (and thus anticipated $\beta_1$) we used a range of assumed values, i.e. $TRA = (25\%, 50\%, 75\%)$. We also varied the number of people in the control

and treatment groups, i.e. $n_0$ and $n_1$. The tables below show the empirical power under these scenarios.

| $TRA = 25\%$ | $n_1$ | | | | | |
|---|---|---|---|---|---|---|
| $n_0$ | 5 | 6 | 7 | 8 | 9 | 10 |
| 10 | 17.0 | 18.4 | 17.4 | 18.4 | 18.2 | 19.0 |
| 11 | 17.2 | 15.8 | 16.6 | 17.6 | 19.6 | 19.4 |
| 12 | 17.0 | 17.6 | 18.2 | 18.4 | 19.4 | 19.2 |
| 13 | 17.2 | 16.4 | 17.6 | 17.4 | 20.6 | 19.6 |
| 14 | 16.6 | 17.8 | 19.8 | 20.2 | 19.4 | 20.2 |
| 15 | 15.4 | 17.4 | 18.8 | 19.4 | 20.8 | 22.6 |
| 16 | 14.6 | 15.2 | 17.8 | 19.8 | 20.4 | 21.0 |
| 17 | 14.4 | 15.8 | 18.6 | 19.0 | 20.6 | 22.4 |
| 18 | 14.4 | 16.2 | 18.2 | 18.8 | 21.0 | 22.6 |
| 19 | 17.4 | 19.0 | 18.6 | 19.4 | 22.2 | 24.8 |
| 20 | 15.8 | 17.8 | 19.4 | 22.2 | 25.8 | 26.8 |

| $TRA = TRA = 50\%$ | $n_1$ | | | | | |
|---|---|---|---|---|---|---|
| $n_0$ | 5 | 6 | 7 | 8 | 9 | 10 |
| 10 | 52.0 | 57.0 | 60.4 | 63.8 | 64.4 | 67.0 |
| 11 | 54.2 | 58.8 | 62.4 | 63.8 | 65.2 | 69.6 |
| 12 | 55.0 | 59.4 | 62.8 | 65.4 | 69.2 | 70.6 |
| 13 | 54.6 | 58.2 | 63.6 | 67.0 | 69.4 | 71.8 |
| 14 | 58.4 | 62.4 | 64.8 | 68.2 | 70.4 | 73.0 |
| 15 | 55.6 | 59.2 | 65.0 | 66.6 | 70.4 | 74.0 |
| 16 | 55.2 | 60.4 | 64.0 | 68.0 | 71.6 | 75.8 |
| 17 | 54.6 | 60.6 | 66.8 | 69.8 | 75.8 | 78.2 |
| 18 | 54.2 | 61.6 | 67.8 | 72.2 | 75.6 | 78.4 |
| 19 | 59.6 | 66.8 | 71.0 | 76.8 | 78.8 | 81.6 |
| 20 | 61.8 | 68.2 | 72.8 | 78.6 | 80.6 | 82.8 |

| $TRA = 75\%$ | $n_1$ | | | | | |
|---|---|---|---|---|---|---|
| $n_0$ | 5 | 6 | 7 | 8 | 9 | 10 |
| 10 | 97.2 | 99.0 | 99.6 | 99.8 | 99.8 | 100.0 |
| 11 | 98.2 | 99.6 | 99.8 | 99.6 | 99.6 | 100.0 |
| 12 | 98.4 | 99.2 | 99.2 | 99.8 | 100.0 | 100.0 |
| 13 | 97.4 | 99.0 | 99.6 | 99.8 | 100.0 | 100.0 |
| 14 | 96.6 | 98.8 | 99.2 | 99.8 | 100.0 | 100.0 |
| 15 | 97.8 | 99.2 | 99.8 | 100.0 | 100.0 | 100.0 |
| 16 | 98.4 | 98.8 | 99.6 | 99.8 | 100.0 | 99.8 |
| 17 | 99.4 | 99.8 | 100.0 | 100.0 | 100.0 | 100.0 |
| 18 | 99.0 | 100.0 | 100.0 | 99.8 | 100.0 | 100.0 |
| 19 | 99.2 | 99.6 | 99.6 | 100.0 | 100.0 | 100.0 |
| 20 | 99.0 | 99.2 | 99.8 | 100.0 | 100.0 | 100.0 |

## Data capture and transfer

Designated trial staff will enter the data required by the protocol into the electronic CRF (eCRF) according to the Data Management Plan. An external monitor will review the data entered into the eCRFs by investigational staff for completeness and accuracy and will instruct the site personnel to make any required corrections or additions. After study close out by the monitor, the eCRF database will be locked by the PI of the study. Data can be exported from the database in Microsoft Excel files for further use in statistical analysis.

### Statistical interim analyses and stopping guidance

There are no pre-defined criteria for study termination in this clinical trial based on SMFA or other biological results. Safety and reactogenicity data will be evaluated by a safety monitoring committee (SMC) after the first R0.6C administration in each group before proceeding to the next group. A summary report of adverse events (AEs) will be provided to the SMC after all groups have completed follow-up (I4+84). The study may be placed on safety hold at any time for the following reasons:

- On advice of the safety monitor;
- On advice of the Principal/Clinical investigators;
- On advice of the SMC;
- On advice of the CCMO;
- If holding rules are met (see below).

If the following holding rules are met, administration of R0.6C will be held for all remaining administrations in that dosage group and subsequent groups:

- One or more participants experience a serious adverse event (SAE) that is determined to be at least possibly related to the administration of R0.6C
- Two or more subjects experience a grade 3 adverse event (local, clinical systemic or laboratory systemic) possibly, probably or definitely related to R0.6C administration and persisting at grade 3 for >72 hours

If considered necessary by the investigators or SMC, remaining administrations in lower dosage groups may also be put on hold.

### Statistical Software

- Safety data analysis will be performed in IBM SPSS (latest version available), or R. To ensure reproducibility an R script or SPSS syntax file will be created.
- SMFA data analysis will be performed using an online data analysis tool developed to estimate transmission reduction in transmission trials with reduction in oocyst density as an endpoint (Add ref: Ramjith, Alkema et al. Frontiers in immunology, accepted for publication) https://bousema-lab.shinyapps.io/transmission_sample_size.
- R0.6C antibody kinetics will be analyzed using standard non-compartmental methods using the Phoenix WinNonlin software package.
- If significant transmission reducing activity is measured in the SMFA, the relationship between serum antibody quantities and transmission reducing activity in SMFA will be assessed using standard compartmental pharmacokinetic models and empirical and mechanistic pharmacokinetic-pharmacodynamic models using the non-linear mixed effects modelling software package NONMEM 7.4, using Piraña 2.9 as an interface and R for data processing.

# Statistical Principles

## Confidence intervals and P values

Two-sided p values less than 0.05 will be considered statistically significant. Adjustments for multiple comparisons will be implemented where appropriate.
Where applicable, normally distributed continuous outcomes will be presented as mean with 95% CI or standard deviations. Continuous outcomes that are not normally distributed will be transformed using a suitable transformation before reporting 95% CIs or will be presented with median and interquartile ranges. For discrete (or count) data we will find interquartile ranges. Binary data will be presented as counts and proportions.

## Adherence and protocol deviations

The SAP will be followed even if data are missing. For the safety analysis, data from all subjects who received at least one dose of R0.6C in the study will be included. Subjects who miss an appointment date will not be removed from the study. Rather, their appointment and laboratory values will be recorded by appropriate missing value notation in the clinical database. Non-analyzable data will be documented in the deviations. A best-worst case sensitivity analysis may be performed.

# Study Outcome Measures

## Outcome Definitions

(Protocol section 8.1).

Primary safety Endpoints:

1) The number of serious adverse events and solicited and unsolicited grade 3 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Primary efficacy endpoints:

2) The functional transmission reducing activity in the standard membrane feeding assay of volunteer sera collected two weeks after the fourth R0.6C immunization (I4+14), compared to baseline (I1-1) within each of the four dose-adjuvant groups.

Secondary safety endpoints:

3) The number of solicited and unsolicited grade 1 and 2 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Secondary efficacy endpoints:

4) The TRA at other timepoints (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84) compared to baseline (I1-1) in each of the four dose-adjuvant groups.

5) The anti-6C antibody quantity in volunteer sera collected two weeks after fourth R0.6C immunization (I4+14) and at other time points (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84)

compared to baseline (I1-1) in each of the four dose-adjuvant combinations, as determined by ELISA.

Exploratory Endpoints:

6) The functional TBA in the SMFA of volunteer sera collected at different time points compared to baseline (I1-1) in each of the four dose-adjuvant combinations.
7) The anti-6C antibody decay rate following R0.6C immunization for each of the four dose-adjuvant combinations.
8) The anti R0.6C and anti-R0 antibody quantity in volunteer sera collected at different time points in each of the four dose-adjuvant combinations.
9) The cellular immune responses in volunteers samples collected at different time points in each of the four dose-adjuvant combinations.
10) The difference in TRA between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).
11) The difference in peak anti-6C antibody quantity between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).

# Analysis Methods

## Trial population

For the safety analysis, data from all subjects who received at least one dose of R0.6C and for whom safety data are available will be included.

### Recruitment

- Recruitment data will be summarized in a flow diagram.

### Screening data

- Screening data will be summarized for each study group. Eligibility criteria are specified in protocol section 5.2 and section 5.3.

### Withdrawal and follow-up

- Procedures for withdrawal of individual subjects are described in protocol section 8.4. Should reasons for withdrawal be known, these will be described, including the timing of withdrawal.

### Baseline patient characteristics

- Demographic data will be summarized by descriptive statistics per dose/adjuvant group and will include the total number of observations (n), plus the mean, standard deviation (SD) and range for normally distributed continuous variables and number and percentages for dichotomous variables. This data will be tabulated.

- Where applicable, continuous outcomes that are not normally distributed will be transformed using a suitable transformation before reporting means and SDs. Where a suitable transformation cannot be found, the mean and SDs will be substituted with the median and interquartile ranges. For discrete (or count) data we will report the medians and interquartile ranges. Binary data will be presented as counts and proportions or percentages.

## Analyses in relation to endpoints

### Safety outcome analyses

- For each solicited (local or general) adverse event type, possibly, probably or definitely related to the R0.6C vaccinations, the number and proportion of subjects experiencing that AE within the protocol-defined timeframe will be tabulated by severity grade for each dose/adjuvant group and for the entire study population. This will also be tabulated for subjects experiencing any solicited local, any solicited general and any solicited AE (see example table below). Where applicable, we will compare these proportions between groups. Relatedness (possibly, probably or definitely) and duration of solicited AEs to R0.6C vaccinations may also be presented. (Endpoints 1 and 3).

- For each unsolicited adverse event type (categorized by ICD-10) that is possibly, probably or definitely related to the R0.6C vaccinations, the number of adverse events per dose group and overall study population will be described categorized by severity grade. Translation from ICD-10 coding to MedDRA terms may be performed where necessary. (Endpoints 1 and 3).

- The proportion of individuals with grade 1, 2 and 3 adverse events may be compared between groups.

- The total number of grade 1, 2 and 3 adverse events as count data may be compared between groups.

- Individual serious adverse events (SAEs) will be summarized, including relatedness to R0.6C vaccinations (Endpoint 1).

- Withdrawals due to AEs/SAEs will be summarized per group.

Table 1 - Descriptive statistics for subjects experiencing solicited AEs

| | | 30µg R0.6C/AlOH | | 30µg R0.6C/AlOH + Matrix-M | | 100µg R0.6C/AlOH | | 100µg R0.6C/AlOH + Matrix-M | | All | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | #Subjects | % | #Subjects | % | #Subjects | % | #Subjects | % | #Subjects | % |
| Local AE1 | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |
| | Grade 3 | | | | | | | | | | |
| | | | | | | | | | | | |
| | Any grade | | | | | | | | | | |
| Local AE2 | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | … | | | | | | | | | | |
| | …. | | | | | | | | | | |
| Any local AE* | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |
| | Grade 3 | | | | | | | | | | |
| | | | | | | | | | | | |
| | Any grade | | | | | | | | | | |
| | | | | | | | | | | | |
| Systemic AE1 | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |
| | … | | | | | | | | | | |
| Any systemic AE* | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |
| | … | | | | | | | | | | |
| | | | | | | | | | | | |
| Any AE* | Grade 1 | | | | | | | | | | |
| | Grade 2 | | | | | | | | | | |
| | … | | | | | | | | | | |

* List (only) highest grade AE per subject

## Clinical Laboratory Data Analysis

- All clinical laboratory abnormalities will be analyzed by participant and will include onset time and duration.

- Any clinically significant deviations in routine laboratory test results, will be reported as unsolicited AEs if they are considered clinically relevant by the investigator.

## Efficacy outcome analyses

- Pfs48/45-6C and R0.6C antibody prevalence and density will be determined by ELISA; the mean of duplicate concentration estimates is used for analyses. Antibody density will be expressed in µg/mL using humanized monoclonal antibody TB31F as a reference/standard. (Endpoint 5, 7 and 11).

- The R0 antibody prevalence and density will be determined by ELISA; the mean of duplicate concentration estimates is used for analyses. Antibody density will be expressed in µg/mL using R0 monoclonal antibody as a reference/standard. Alternatively, R0 antibody densities may be expressed in arbitrary units (AU) or R0 antibody densities may be estimated by subtracting measured R0 antibody levels from R0.6C antibody levels. (Endpoint 8).

- Pfs48/45-6C antibody density will be plotted over time per individual and either as a mean or geometric mean per group. AUC and mixed effects models may be used to investigate the effect of timepoints, dose/adjuvant group and their interactions on the 6C antibody concentration. Mixed models use specific random intercepts to control for the correlation within individuals (Endpoint 7).

- TRA will be quantified as the relative reduction in oocyst intensity for test samples (one feeder per test sample) compared to baseline serum controls. Samples may be tested in one or two independent SMFA experiments. If significant transmission reduction is measured, TRA values for each participant and time-point will be estimated using generalized linear models, as previously described[1]. (Endpoint 2, 4 and 10).

- We may calculate the percentage of individuals per group that have at least 50%, 80% and 90% TRA at relevant timepoints and the percentage of individuals per group with statistically significant TRA at relevant timepoints. Mean TRA levels may also be calculated per study group per time point and compared between groups. (Endpoint 10).

- Modeled TRA estimates (based on antibody concentration) may be plotted against observed TRA estimates.

- TBA will be quantified as the reduction in oocyst prevalence in the presence of test serum as compared to (pooled) baseline serum[1] and presented per group and time-point. (Endpoint 6).

# References

1. Churcher TS, Blagborough AM, Delves M, et al. Measuring the blockade of malaria transmission--an analysis of the Standard Membrane Feeding Assay. *Int J Parasitol* 2012; **42**(11): 1037-44.