Letter of Intent - Biomarker Qualification

Submission title: ***Plasmodium 18S rRNA/rDNA biomarker for controlled human malaria infection studies in malaria-endemic regions***

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1. **Drug Development Need Statement**
   - **a.** The malaria pipeline contains an increasing number of drug and vaccine candidates. Although thick blood smears (TBS) long served as a gold standard for detection of *Plasmodium* infection in human blood, more sensitive molecular tests have been developed. The University of Washington (Point of contact: Sean C. Murphy, MD/PhD) worked with FDA from 2014-2018 to qualify the *Plasmodium* 18S rRNA/rDNA biomarker to replace blood smears in controlled human malaria infection (CHMI) studies conducted *in non-endemic sites* (DDTBMQ000044). This biomarker achieves earlier and more analytically sensitive detection of *Plasmodium falciparum* infections than TBS and is qualified for use in initiating treatment of participants post-challenge. The non-endemic COU Qualification Package portfolio provides extensive history and rationale for the *Plasmodium* 18S rRNA/rDNA biomarker, and we refer FDA reviewers to that DDTBMQ000044 submission for historical and technical context. In addition to the clinical validation information, the analytical validation of the reverse transcription polymerase chain reaction (RT-PCR) used for biomarker detection were also extensively reviewed by the FDA DDT Biomarker Qualification review team for the qualified DDTBMQ000044 COU.
   - **b.** This new LOI proposes to build on the non-endemic CHMI COU by extending the COU to CHMI at endemic sites. The endemic CHMI COU submission will utilize most of the same analyses performed for the non-endemic site COU using samples from studies conducted in endemic settings. These analyses may additionally identify differences unique to the endemic site CHMI COU. Some these differences are noted below.
   - **c.** As seen in non-endemic sites, use of the proposed biomarker in endemic CHMI studies will likely improve TBS endpoints by providing more sensitive, more specific, and more quantitative data to inform study endpoints. The data will be analyzed to determine how the biomarker identifies blood-stage infections in comparison to TBS and will be analyzed to demonstrate whether this provides an actionable, quantitative treatment threshold post-CHMI (just like the biomarker is used in the qualified non-endemic CHMI COU). In addition, the biomarker may also detect subpatent CHMI infections that emerge from the liver into the blood but do not achieve a patent parasite density. Such infections can be suppressed by anti-erythrocyte stage antibody responses acquired by persons in endemic regions due to repeated natural exposures to *Plasmodium* parasites. Biomarker detection of subpatent infections may demonstrate that CHMI procedures were successful even if pre-existing immune responses subsequently modify the growth of such infections. This information could help better understand the difference between vaccine or drug treatment groups (especially between placebo control persons who are usually expected to become infected and candidate vaccine/drug group persons who might not).
   - **d.** Another important difference between non-endemic and endemic COUs is that there is a greater risk of pre-existing *Plasmodium* infection at endemic sites than non-endemic sites. In endemic site studies, a variety of drug clearance approaches have been taken during activities leading up to challenge. During the vaccination phase for instance, some studies have treated participants with one or more anti-malaria drugs to clear existing parasites whereas other studies have not included vaccination-phase drug treatment.

Studies have been done in areas of varying endemicity, making the need for vaccination phase treatment somewhat site-specific. As a study approaches its CHMI date, drug clearance stops being an option because the half-life of anti-malarial drugs may interfere with the CHMI itself. On or just prior to the date of CHMI, a blood sample is always obtained and can be used to determine if the person was infected or not *prior to* challenge. In our work, we will systematically compare pre-CHMI samples to post-CHMI samples. During CHMI studies in endemic regions, participants are usually domiciled to reduce the risk of acquiring a non-challenge strain infection post-CHMI. Thus, in persons who are biomarker-negative at the time of challenge and then become biomarker-positive thereafter, it is reasonable to conclude that biomarker was derived from challenge strain parasites. In the event that biomarker indicative of a pre-existing non-*P. falciparum* infection is found in the pre-CHMI sample, these infections can be easily discerned from *P. falciparum* infections since they would be positive for pan-*Plasmodium* regions of the biomarker, but not for *P. falciparum*-specific regions of the biomarker. Assays designed to detect both targets (such as the assay used in the DDTBMQ000044 submission and proposes herein) can be leveraged to differentiate between *P. falciparum* and non-*P. falciparum* infections to clearly inform the selected endpoints. In the event that biomarker indicative of a pre-existing *P. falciparum* infection is found in the pre-CHMI sample and the participant stays biomarker positive post-CHMI, additional tools like Sanger sequencing and next-generation sequencing could be brought to differentiate challenge strain parasites from field-acquired parasites.

2. **Biomarker Information and Interpretation**
   a. ***Biomarker name***: *Plasmodium* 18S rRNA/rDNA
   b. ***Analytical methods***: RNA or total nucleic acid extraction followed by quantitative reverse transcription PCR (qRT-PCR). For this effort, we will utilize an assay based on the assay presented in DDTBMQ000044. It may also be possible to use appropriately designed 18S rDNA PCR-only assays (as is performed by several other CHMI centers).
      i. Pan-*Plasmodium* 18S rRNA (with Armored RNA calibrator in whole blood)
      ii. *P. falciparum*-specific 18S rRNA (with Armored RNA calibrator in whole blood)
      iii. Host mRNA or rRNA control target
   c. ***Measurement units and limit(s) of detection***:
      i. Copies/mL of whole blood (LOD 5.3×10^4 copies/mL whole blood)
      ii. Estimated parasites/mL of blood (if *P. falciparum*) (LOD 20 estimated parasites/mL)
   d. ***Biomarker interpretation and utility***:
      i. Interpretation is as in DDTBMQ000044. Briefly, the biomarker assay provides copies/mL of the qRT-PCR target sequences (for pan-*Plasmodium* and *P. falciparum* targets). These copies/mL values are reported to the study team. In addition, we determined a conversion factor for *P. falciparum* ($7.4\text{x}10^3$ copies/ring stage *P. falciparum* parasite) to provide an 'estimated' parasites/mL of whole blood value. This estimated parasites/mL density has been found to agree with DNA-based measures of parasite density (where the target number is genomically fixed). This conversion factor was extensively discussed with FDA

in our previous submission. Thus, we report a copies/mL value and an estimated parasites/mL value. It is our strong recommendation that parasite density estimates help align trial results with historical trials and studies tested by other endpoints.

ii. In the qualified non-endemic CHMI COU, the therapeutic intervention (anti-malarial treatment) in response to the biomarker is intended to be set individually for each clinical trial protocol. This means that investigators who use the biomarker would specify in their protocol a biomarker-based definition that would be used to initiate treatment. This threshold may be different for studies with different types of products or different reasons for doing the study. Usually this threshold would be selected to reduce symptoms and accelerate infection detection compared to TBS. For instance, the protocol could one positive biomarker result over a defined threshold (e.g., >1.85×10^6 copies/mL, equivalent to ~250 estimated *P. falciparum* parasites/mL).

iii. Reportable range: 1.48×10^5 - 7.4×10^{10} copies/mL (equivalent to 2×10^1 to 1×10^7 estimated *P. falciparum* parasites/mL (note that the approximate LOD of TBS is 5×10^3 to 2×10^4 parasites/mL).

3. Context of Use Statement

a. A monitoring biomarker, that when positive, informs initiation of treatment with an anti-malarial drug >6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoites in healthy subjects (18-45 years old) from endemic areas enrolled in clinical studies for vaccine and/or drug development.

4. Analytical Considerations

a. ***General description of biomarker being measured and methods***: The biomarker assay is a standard qRT-PCR for 100-350 bp long regions within the 2.1 kb 18S rRNA sequences of *Plasmodium* parasites. The amount of 18S rRNA biomarker is an indicator of total parasite burden. The assay is substantially equivalent to the assay presented in DDTBMQ000044 with slight modifications intended to further increase analytical specificity.

b. ***Brief description of sample source***: Whole blood collected in EDTA anticoagulant. Typical volume is 50 µL of blood.

c. Description of pre-analytical factors and quality assurance/quality control (QA/QC) plans to preserve specimen integrity:

i. ***Liquid whole blood samples***: Preferably aliquoted into bioMerieux NucliSENS lysis buffer (guanidinium-based lysis buffer) and frozen at ≤-70°C. Alternatively can be frozen as-is as whole blood at ≤-70°C. The biomarker has been shown to be stable under these conditions and this data was reported in DDTBMQ000044.

ii. ***Control samples*** (run controls): cultured, ring-synchronized *P. falciparum* parasites added to whole blood and then prepared as above as liquid samples. Run control performance is monitored by Levey-Jennings plots following each run (high, low, negative) as in DDTBMQ000044.

iii. ***Calibration***: achieved by an Assuragen Armored RNA calibrator that encodes the full-length *P. falciparum* 18S rRNA and provides traceable quantification as in DDTBMQ000044.

d. Analytical validation plan

i. The original biomarker detection assay was tested for accuracy, precision, analytical sensitivity, sample stability and analytical specificity (interferences), reportable range, and carryover using whole blood samples and was provided to FDA for liquid venous blood samples under DDTBMQ000044. The assay has since been refined slightly to further increase analytical specificity (see changes in non-public attachment). Data showing substantial equivalence between the revised assay and that reviewed under DDTBMQ000044 will be submitted for this endemic CHMI COU.

5. Clinical Considerations

a. Precedent for biomarker use in endemic site CHMI studies

i. There is excellent precedent for the qualification of this biomarker for endemic site CHMI studies. To date, clinicaltrials.gov lists following trials as completed or ongoing CHMI trials in malaria-endemic sites in Africa that have used TBS and/or *Plasmodium* 18S rRNA/rDNA-based molecular endpoints (**Table 1**).

Table 1. CHMI trials in endemic regions with TBS and molecular biomarker endpoints

Clinicaltrials.gov	Study title	Location; # participants & eligibility; relevant endpoints; status
NCT03496454	Susceptibility of Gambian Adults to PfSPZ-Challenge Infection in the Controlled Human Malaria Infection Model (CHMI-Gambia1)	The Gambia; 19 healthy male participants aged 18-35 yrs; TBS and qPCR; completed
NCT03590340	Regimen Optimization Trial of PfSPZ Vaccine in Equatorial Guinea (EGSPZV3)	Equatorial Guinea; 104 estimated male and female participants aged 18-45 yrs; TBS and qPCR; completed
NCT02859350	Safety, Tolerability and Immunogenicity of PfSPZ Vaccine in an Age De-escalation Trial in Equatorial Guinea (EGSPZV2)	Equatorial Guinea; 135 estimated healthy adults in CVac group vs controls; TBS and qPCR; completed
NCT02237586	Effect of Plasmodium Falciparum Exposure and Sickle Cell Trait on Infection Rates and Kinetics After IV Administration of PfSPZ Challenge	Gabon; 25 non-healthy participants 18-30 years (AA, AS, SS phenotypes); TBS and qPCR; completed
NCT03420053	Study to Evaluate Safety, Immunogenicity and Efficacy of PfSPZ Vaccine in HIV Negative and HIV Positive Tanzanian Adults (BSPZV3a)	Tanzania; 21 adults including some HIV+ persons; TBS and qPCR; completed
NCT02613520	Safety and Immunogenicity in Age De-Escalation of PfSPZ Vaccine in Tanzanian Adults, Children, and Infants	Tanzania; 105 participants with only healthy adults to CHMI; TBS and qPCR; completed
NCT01540903	Controlled Human Malaria Infection by Intradermal Injection of Plasmodium Falciparum Sporozoites in Tanzanian	Tanzania; 30 healthy male participants 20-35 years old; TBS and qPCR; completed

	Adults	
NCT02627456	Safety, Immunogenicity, and Protective Efficacy of Radiation Attenuated Plasmodium Falciparum NF54 Sporozoites (PfSPZ Vaccine) in Healthy African Adults in Mali	Mali; 415 participants; TBS; completed

a. ***How will the biomarker measurement inform drug development?***

   ii. As proposed herein, the biomarker is intended to tell investigators when an erythrocyte-stage *P. falciparum* infection has developed post-CHMI. For the proposed COU, this information is used to determine when to initiate treatment to cure the CHMI-initiated infection. Thus, this is primarily a safety endpoint.

   iii. The information may also be useful for determining if and when a *Plasmodium* infection was present in the blood as an efficacy.

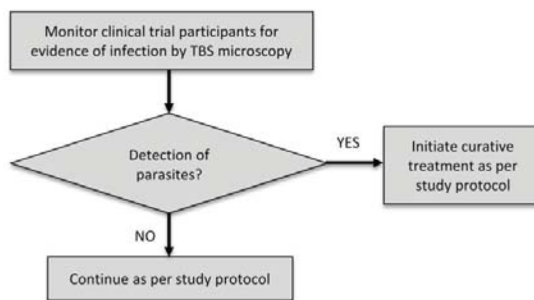
   iv. With respect to drug/vaccine development, biomarker positivity in a participant that received an investigational product intended to prevent post-CHMI erythrocyte stage infection could indicate that the product did not achieve complete pre-erythrocytic protection. However, since the vaccine and drug products may vary, how the biomarker will be used to inform efficacy endpoints would be contained in each clinical trial protocol.

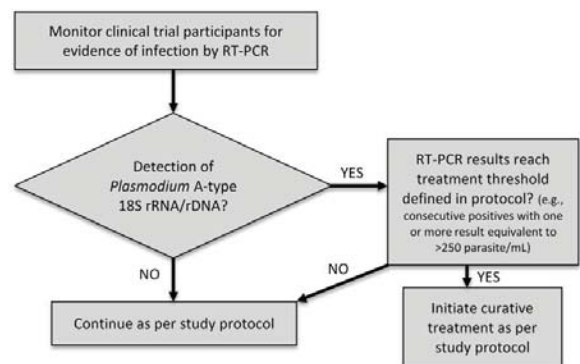
   v. These clinical considerations are similar to those addressed in DDTBMQ000044.

b. ***Decision tree***

   i. The decision tree for this endemic site CHMI COU (**Figure 1**) is the same as for the non-endemic CHMI COU in DDTBMQ000044.

Figure 1. Decision trees for TBS microscopy (A) and 18S rRNA/rDNA biomarker detection (B). The treatment threshold listed in B is an example; it may vary from study-to-study and would be defined in the study-specific protocol based on the specific study design. This is the same decision tree as listed in DDTBMQ000044.





c. ***Patient population***: CHMI studies enroll healthy, adult volunteers, usually aged 18 to 45 years old. The inclusion/exclusion criteria are not significantly different than CHMI studies in non-endemic sites as submitted in Appendix 2 of DDTBMQ000044.

d. ***Setting***: CHMI studies in malaria-endemic countries (examples shown in Table 1).

e. ***Clinical validation***: The study design will be equivalent to that used for the non-endemic qualification DDTBMQ000044. Key aspects are briefly summarized below.

i. ***Format of data***: Clinical validation data will be provided in the same format as in DDTBMQ000044 (Qualification Package with Appendicies including Subject-level Data in .sas format) to support the biological and clinical relevance of the biomarker for the proposed endemic CHMI COU.

ii. ***Reference values***: As in the non-endeimc CHMI COU, the ideal reference interval of the healthy, normal population is "not detected". As noted above, the pre-CHMI and post-CHMI samples can be compared to determine if this was the case for enrolled participants. Since the biomarker assay is more sensitive than TBS, it is likely that we will find some persons with low density *Plasmodium* infections prior to challenge. Thus, this endemic site qualification study will determine if low density biomarker positivity is a common or rare occurrence prior to CHMI.

iii. ***Proposed clinical validation:***

1. **Literature review for endemic site studies:** Existing data from the peer-reviewed literature on endemic site CHMI sites will be provided to support the proposed COU as in DDTBMQ000044.
2. **Data from prospective testing of endemic CHMI studies**
    a. Using the analytically-validated venous blood SOP, we will obtain archival blood samples from several of the CHMI trials listed in Table 1. These samples will be tested by the UW *Plasmodium* 18S rRNA qRT-PCR and compared against the TBS results that served as the treatment endpoint in these studies. These data will be evaluated on a cohort-by-cohort basis, on a trial-by-trial basis, and on a composite basis across all trials. We will assess daily concordance between biomarker and TBS as well as overall concordance (e.g., Does a biomarker-positive person eventually go on to become TBS-positive as well?). This approach is the same as that taken in DDTBMQ000044.

    b. We will determine the mean number of days (±95%CI) from CHMI to any biomarker positivity, to different thresholds of biomarker positivity, to any Grade 1, 2 or 3 symptoms (if any), and to TBS positivity (if any). We will determine which of these differences are statistically significant using t-tests. We will also determine the mean copy number and mean estimated parasite density (±95%CI) for the first biomarker positive sample and at the time of TBS positivity. We will evaluate whether different biomarker-based treatment thresholds are likely to reduce symptoms. These comparisons are consistent with the approaches taken in DDTBMQ000044.

    c. We will also determine whether biomarker data recapitulates conclusions about group differences originally made on the basis of TBS.

    d. We will also compare to available biomarker data from outside laboratories (where available) to perform discrepant analysis in

situations that are qRT-PCR biomarker-positive/TBS-negative. This analysis will follow FDA guidance on this topic and is consistent with the discrepant analysis performed in DDTBMQ000044.

e. Special additional analyses may be undertaken to address issues more likely in endemic settings such as evaluations of biomarker positivity prior to CHMI or the presence of non-*P. falciparum* parasites.

iv. ***Benefits and Risks of applying the biomarker in drug development or a clinical trial:*** As in Table 2 of the Biomarker Qualification for DDTBMQ000044. In addition, a biomarker-based approach may have adidtional benefits in endemic sites since the biomarker-based approach may be able to detect scientifically meaningful blood-stage infections that would otherwise be missed by TBS.

v. **Current knowledge gaps, limitations and assumptions for the proposed COU**: The biggest knowledge gap pertaining to this proposed endemic CHMI COU is that we do not know how asymptomatic infection varies across the malaria-endemic world. We presume that asymptomatic infection modified immune responses and serves to periodically or continuously boost the immune system and to mitigate severe disease signs and symptoms. However, we do not know how asymptomatic infections are geographically dispersed because, until recently, relatively insensitive methods have been used for field prevalence surveys. By testing a subset of samples derived from studies in Table 1, we will be able to rigorously evaluate the biomarker its potential to fulfill the proposed endemic CHMI COU.

6. Supporting Information

a. The supporting evidence for a link between biomarker positivity and true TBS-defined malaria infection are fully explained in DDTBMQ000044 and in the attached paper by Seilie, Chang et al (Attachment 1).

b. The Qualification Letter for DDTBMQ000044 is included (Attachment 2).

c. The specific COU of endemic sites will be addressed by performing biomarker testing and then data analyses from the endemic CHMI studies described above. These studies will ascertain whether the findings in DDTBMQ000044 apply equally well to endemic CHMI studies. The enrolled studies from endemic regions will contain a TBS gold standard comparator.

7. Previous Qualification Interactions and Other Approvals

a. Our non-endemic site CHMI COU was qualified in October 2018 under DDTBMQ000044 (Attachment 2).

b. During our interactions with FDA, we have discussed our plans to expand the qualified COU to include endemic site COUs. This LOI concerns the endemic CHMI COU.

c. This COU is part of a process of COU expansion for the *Plasmodium* 18S rRNA/rDNA biomarker for a wider array of drug and vaccine trials globally. Beyond its use in non-endemic and endemic CHMI studies, we also hope to qualify the biomarker for use in

field studies of naturally-acquired *Plasmodium* infection. Such studies could include Phase 2-3 studies of drugs and vaccines as well as other studies where more indirect public health interventions may have been deployed. Therefore, we will also be submitting a parallel COU for endemic sites monitoring for naturally-acquired *Plasmodium* infection (DDT Tracking number not yet assigned).

8. List of Attachments

a. Attachment 1: Seilie, Chang et al. 2019. Beyond Blood Smears. *Amer J Trop Med Hyg*. (this paper describes the findings from DDTBMQ000044)

b. Attachment 2: Non-endemic site CHMI COU Biomarker Qualification Letter