U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

BIOMARKER QUALIFICATION

Biomarker Name	*Plasmodium falciparum* 18S ribosomal (r)RNA/rDNA
Context of Use*	A type A monitoring biomarker to inform initiation of rescue treatment with an anti-malarial drug at least 6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoites in healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against *P. falciparum*
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Briefing Documents Date(s)	March 15, 2017
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Keywords: Drug, Monitoring Biomarker, Safety Biomarker, Vaccine

*FDA Biomarker Qualification Review Team Consensus Context of Use

The Proposed Context of Use (CoU) and Condition of Use for *Plasmodium* 18S rRNA/rDNA biomarker

- **General Area**

    - **Requester's proposed CoU**

      Detection of the *P. falciparum* 18S rRNA/rDNA is a safety (and efficacy) endpoint for initiating treatment before clinical malaria symptoms appear in subjects who have undergone *P. falciparum* sporozoite controlled human malaria infection (CHMI) in non-endemic regions

    - **Biomarker Qualification Review Team's Consensus During the Review Stage**

      A monitoring biomarker to inform initiation of rescue treatment with an anti-malarial drug at least 6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoites in healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against *P. falciparum*

      The biomarker can be tested for at ≥ 6 days post-CHMI in human whole blood. The *P. falciparum* 18S rRNA/rDNA biomarker must have been measured with one or more specific nucleic acid amplification-based methods. This biomarker is intended to replace the use of thick blood smear (TBS) microscopy for this endpoint."

- **Target Population for Use**

  Healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against *P. falciparum*

- **Stage of drug development for use**

  Early phase, e.g., proof of concept study, drug development or vaccine development

- **Intended application**

  In CHMI studies – healthy, malaria-naïve volunteers aged 18 through 45-50 years old. Subjects are expected to be negative for the biomarker at baseline

Assay used to assess clinical specimens for qualification review

- The University of Washington (UW) second generation *P. falciparum* 18S rRNA/rDNA biomarker reverse transcriptase (RT)-PCR assay
- The UW third generation Pan-*Plasmodium*/*P. falciparum* 18S rRNA/rDNA biomarker RT-PCR assay
- Thick blood smear (TBS)

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I. Executive Summary

Human infectivity controlled malaria infection subjects from Studies MC-001 DEMO, MC-003 ITV and PfSPZ-CVac were evaluated individually and across the studies. Comparing to the TBS criterion, the current gold standard, the results of this reviewer's time to positivity analyses consistently showed a shorter time to positivity with the proposed PCR biomarker using either any PCR positivity or at least 250 estimated parasites/mL criteria.

In subjects receiving drug or vaccine, depending on the type of intervention received, time to positivity analysis was performed by individual study and across the studies. Like what was observed in the infectivity control subjects, the proposed PCR biomarker consistently showed a shorter median time to positivity than TBS gold standard assay. There was a caveat. Of the three clinical studies, there was one false negative call with PCR assay in subject (b) (6) in MC-003 IVT study. The ability of PCR assay to show a shorter time to PCR positivity than time to TBS positivity became less clear statistically in MC-003 IVT study. However, the estimated median time to positivity with PCR appeared to be still shorter than that with TBS criterion. The false negative occurred approximately 2.5% to 3% in the treated or vaccinated subjects including versus excluding drug control subjects evaluated. When all treated or vaccinated subjects time to positivity were pooled, the overall median time to positivity was shorter with PCR biomarker than with TBS assay.

It is worth noting that in the drug control arm only studied in MC-003 ITV study, the median time to positivity with PCR biomarker was shorter by approximately 2 days than median time to TBS positivity. The median time to first clinical sign or symptom was approximately 1 day shorter than median time to TBS positivity.

The criterion of any PCR positivity and the criterion of at least 250 estimated parasites/mL were each shown to accelerate earlier detection of positivity for malaria. The data support the conclusion that PCR biomarker is superior than TBS assay and can be a replacement of gold standard TBS assay. We note that the sample size in the individual study were small. It is understood that the sample size is generally small for early phase drug or vaccine development. More data should be collected when the PCR biomarker is used as a tool for drug or vaccine development.

We recommend use of any PCR positivity as the criterion to monitor and inform initiation of rescue treatment with an anti-malarial drug at least 6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoits in heathy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against *P. falciparum*. The criteria have a better chance of early detection and are ethically sound given CHMI study enrolls healthy volunteers. The ability of earlier detection can also encourage normal healthy subjects to volunteer for CHMI study, which can accelerate drug or vaccine development in treating or preventing Malaria.

II. Milestone Timelines of the CDER Biomarker Qualification Submission

12/04/2014: Dr. Murphy submits Letter of Intent (LOI) to FDA
05/13/2015: FDA responded
09/10/2015: Dr. Murphy submitted initial briefing documents

11/30/2015: face-to-face meeting
02/01/2016: summary of face-to-face meeting
02/22/2017: FDA's invitation letter for full qualification application (FQP) submission
03/15/2017: FDA received Dr. Murphy's FQP
05/04/2017: FDA internal meeting to discuss initial impressions of FQP
08/08/2017: Dr. Murphy's response to FDA IR in June 2017

III. Review of Requester's Proposed Context of Use

In this review, "*Plasmodium falciparum* 18S ribosomal (r)RNA/rDNA A type" biomarker will be referred to as "*P. falciparum* 18S rRNA/rDNA" biomarker. During the consultation and advice stage, the requester was informed that the proposed context of use of *P. falciparum* 18S rRNA/rDNA biomarker should be limited to safety scope for starting rescue treatment early before clinical malaria symptoms appear in subjects post-CHMI (controlled human malaria infection) using *P. falciparum* sporozoites. The rationales for this consideration were articulated by Dr. Chattopadhyay of CBER. That is, FDA requested the requester to clarify "whether the threshold for biomarker detection and parasite density estimation will be same for both efficacy and the safety (which is the decision point for initiating rescue drug treatment for the subjects) endpoints of 'novel' malaria vaccines and drugs under study. For example, if the protocol specifies the safety endpoint (i.e., the trigger to start rescue anti-malarial drug treatment) is 20 estimated parasites/μL blood on day 7 post-CHMI, will the efficacy endpoint of the novel malaria vaccine or drug under the study be set at ≤ 20 estimated parasites/μL on Day 7 post-CHMI too?"

The key concern is whether use of *P. falciparum* 18S rRNA/rDNA biomarker will be based on an estimated parasitemias ≥20 parasites/μL for consideration of an ineffective novel vaccine or drug. If a rescue treatment will be initiated at 20 estimated parasites/μL on Day 7 post-CHMI, but, the efficacy endpoint for the novel malaria vaccine or drug under the study will also be pre-specified at ≥ 20 estimated parasites/μL, the requester was informed that such consideration will be outside the scope of this biomarker qualification review.

However, the requester did not address the above issue in their full qualification package (FQP). Therefore, the proposed COU appears inappropriate. The multi-disciplinary biomarker review team (BQRT) met to discuss what would be an appropriate context of use during the review stage. Two potential contexts of use were discussed: diagnostic biomarker versus monitoring biomarker.

Based on the recently published BEST resource, a diagnostic biomarker is biomarker used to detect or confirm presence of a disease or condition of interest or to identify individuals with a subtype of the disease. In contrast, a monitoring biomarker is a biomarker measured serially for assessing status of a disease or medical condition or for evidence of exposure to (or effect of) a medical product or an environmental agent. The BEST glossary can be found in https://www.ncbi.nlm.nih.gov/books/NBK338448/

To consider *P. falciparum* 18S rRNA/rDNA biomarker as a diagnostic biomarker, the potential applicability would be detection of *P. falciparum* 18S rRNA/rDNA. However, biomarker measurements were collected over a course of up to 28 days in the CHMI studies, whether the measurements are presence/absence using the conventional TBS assay or the amount of *P.*

falciparum 18S rRNA/rDNA using the rtPCR assays compared to a threshold. Given the biomarker data were collected serially for assessing evidence of infection for consideration of whether or not to employ rescue medication, the BQRT reached the consensus that the COU should be to 'monitor' for evidence of infection.

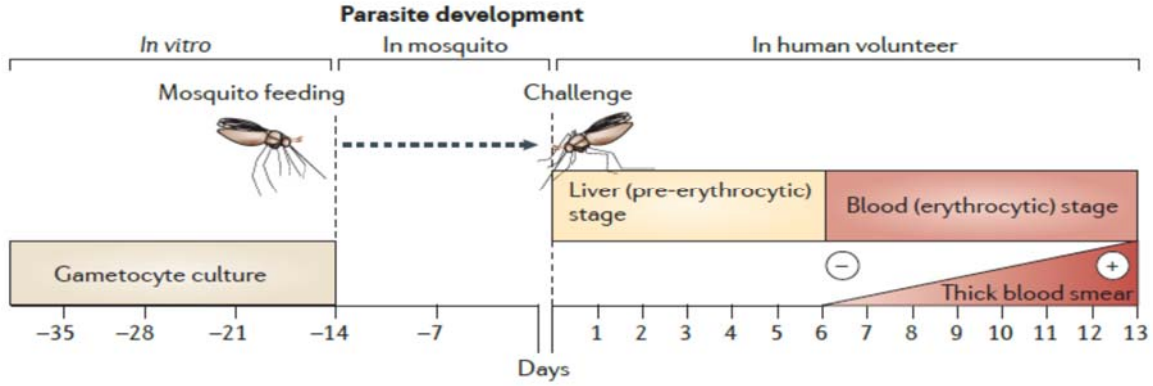
In what follows, this review evaluates evidence based on the consensus COU. Namely, the appropriate COU for P. falciparum 18S rRNA/rDNA is *a monitoring biomarker to inform initiation of rescue treatment with an anti-malarial drug at least 6 days following controlled human malaria infection (CHMI) with P. falciparum sporozoites in healthy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against P. falciparum.*

IV. Assessments of Biomarker's Clinical Utility

1. Background

Published supporting data for this BQ package included 26 published studies encompassing 761 CHMI subjects. The requester described the *Plasmodium* sporozoite CHMI model using Figure 1 presented as the timeline of *P. falciparum* sporozoite challenge infection in human.

Figure 1. Timeline of *P. falciparum* sporozoite challenge infection in humans*



*Extracted from Figure 2 of Sauerwein RW, Roestenberg M, Moorthy VS (2011, Nature Review, Immunology).

In the FQP submission, the sporozoite CHMI model begins with Anopheles mosquitoes acquiring *P. falciparum* gametocytes by membrane feeding on in vitro parasite cultures in donor blood and being reared under GMP-qualified conditions until salivary gland sporozoites develop. In general, eligible subjects receive the designated number of infected bites on day 0 and remain asymptomatic during the pre-erythrocytic stage, referred to as mosquito bite CHMI. In lieu of mosquito bites, direct venous injection (DVI) of cryopreserved parasites is also possible on day 0, referred to as 3200 PfSPZ-DVI CHMI. On or after day 6, parasites emerge from hepatocytes and initiate cyclical red blood cell infections that culminate in eventual detection by biomarker assay or thick blood smear (TBS). Sauerwein et al. (2011) explained that parasites can be detected in the blood of unprotected volunteers by microscopy (using a TBS) on average 11 days (range 7-15 days) after challenge.

After infection, subjects are monitored closely. Signs and symptoms such as abdominal pain, arthralgia, chest pain, chills, diarrhea, fever, headache, low back pain, malaise, myalgia, nausea, temperature continuing, vomiting are collected mostly from post-CHMI day 5-6 to day 18-19 and day 28. The *P. falciparum* S18 rRNA/rDNA biomarker data measured by number of estimated parasite density/mL, biomarker cycle threshold (Ct) value, number of copies/mL of blood (log10), in addition to the estimated number of days CHMI to (i) any positive qRT-PCR, (ii) ≥ 20 parasites/mL, (iii) ≥ 100 parasites/mL, (iv) ≥ 250 parasites/mL, (v) ≥ 1000 parasites/mL, and (vi) ≥10,000 parasites/mL, were also collected for the clinical studies.

From the requester FQP, the assay performed at the UW has evolved over time; the clinical specimens were tested by either the 2nd or 3rd generation assays. The results of the 2nd and the 3rd generation assays were based on the *P. falciparum* and pan-*Plasmodium* channels, respectively. Some of the other differences between the 2nd generation and 3rd generation assays include differences in primers used, internal control, thermocycling program, the conversion factor used for converting number of copies/mL to estimate parasite equivalent/mL. Conversion factor for the 2nd generation assay was 3500 whereas for the 3rd generation assay was 7400. According to the requester, the differences in the conversion factor are due to the nucleic acid recovery and mastermix performance as these are platform and assay specific.

The requester claims that the UW RT-PCR assay is more sensitive than TBS. The requester noted that the published literature supports the proposed COU to accelerate infection detection and reduce malaria-related symptoms in CHMI studies. The requester also noted that the published data strongly supports the ability of the biomarker to differentiate between fully-protected and unprotected subjects. Less published data is available regarding the sensitivity of the approach for detecting partial protection using a biomarker as compared to TBS time to positivity (TTP).

There are four clinical studies enrolling subjects 18-50 years of age. Raw clinical data, biomarker testing and thick blood smear (TBS) results were collected from each clinical study. Malaria-related clinical symptoms including fever, malaise, headache, nausea, vomiting, chills, low back pain, diarrhea, abdominal pain, arthralgia and chest pain were also collected. Table 1, extracted from Table 12 of the requester's FQP package, gave high level summary of each clinical study. Details of each study design can be found in the joint review (uni-review) by Drs. Shukal Bala, Rana Chattopadhyay, and Sue-Jane Wang.

2. Clinical Studies

The requester protocols and results of four CHMI studies (MC-001 DEMO, MC-003 ITV, PfSPZ-CVac PYR, and GAP3KO) were included (Table 1). Testing for the biomarker was performed by RT-PCR assay at the UW using stored whole blood samples. Although the requester referred to 26 published studies to support the qualification of the biomarker, it is worth noting that the assays used in the published studies are different from the assay used by the requester. The details of the assays from these published studies were insufficient for review.

Table 1. Studies included in this BQ**

Study	# subjects at CHMI	CHMI Route & Dose	# subjects TBS+	# subjects biomarker+	# subjects treated for breakthrough infection
MC-001	6	5-bite	6	6	6
MC-003	29	5 bite	26	25	26
PfSPZ-CVac PYR	21	3200 DVI	6	15	15
GAP3KO	10*	200 bite*	0	0	0

Administration of experimental GAP3KO product (not a wild-type CHMI)

**Extracted from Table 12 (p.53 of 118 pages) of the Requester's package

This review focuses on three clinical studies that all include infectivity control subjects whose infection CHMI route was either 5 mosquito bites (5-bite) or 3200 direct venous inoculation (DVI). Study GAP3KO had a different CHMI route with administration of experimental GAP3KO product that was not a wild-type CHMI. Study GAP3KO will not be evaluated in this review. Plasmodium 18S rRNA/rDNA biomarker was measured by RT-PCR assay. For the purpose of the primary biomarker qualification, UW RT-PCR (referred to as PCR) biomarker positivity was defined as positive results in the pan-Plasmodium channel. Clinical endpoints and laboratory endpoints included for evaluation are

TBS: positive/negative; time to TBS positivity

- PCR biomarker: positive/negative; time to PCR biomarker positivity; any positive estimated parasite density at first positive, at least 250 estimated parasite/mL
    - Tested by rt-PCR at University of Washington
    - Tested by rt-PCR or PCR by outside laboratories

- Malaria-related symptoms: time to any symptom

Several small group reviewer meetings among biologists from CBER and CDRH, the microbiologist from CDER and the statistician (myself) from CDER took place during the review of this qualification submission for a coordinated review and to better frame the key questions for evaluation. Assuming that the second generation RT-PCR assay is comparable to the third generation RT-PCR assay, this reviewer performed time to positivity type analysis to assess whether time to positivity overall was shorter with PCR defined by (i) or (ii) below than with time to TBS positivity

(i) any PCR positivity
(ii) at least 250 estimated parasites/mL positivity

This reviewer also performed a time to positivity analysis exploring the time to first clinical sign or symptom in comparison with (i), (ii) and TBS positivity to better understand the timing of any first clinical sign or symptom. Two types of censoring were employed in the time to positivity analysis. Subjects who did not develop positivity from the assay were assigned a relatively more extreme censoring. That is, the censoring day was at 28 days when the CHMI study ended. A more realistic censoring was explored by assigning subjects who did not develop positivity as being censored at the number of days CHMI to rescue treatment. When there was no censoring, the result of these two analyses was the same. In such cases, only one type of censoring analysis

result is given. In this section, this reviewer's analysis results are presented by intervention type for each individual study.

2.1 MC-001 DEMO

MC-001 DEMO was a single-center, open-label, phase I clinical study to demonstrate mosquito bite CHMI under an IND and obtain immunological endpoints after a single exposure. Six healthy subjects volunteered for the CHMI study.

Table 2.1 Time to positivity analysis censored at 28 days – MC-001 DEMO Study

n=6 infective control subjects		Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)		11 (9, 14)	7 (7, 10)	9 (7, 12)	11.5 (10, 14)
% censor		0%	0%	0%	0%
Nominal p-value		0.0004*			

*global test

Although only 6 infected control subjects were studied, a clear signal of a shorter median time to PCR positivity than to TBS positivity is shown in Table 2.1, i.e., approximately 2 days earlier using at least 250 estimated parasites/mL criterion and approximately 4 days earlier using any PCR positivity criterion. The time to the first clinical sign or symptom appeared to be similar to that with time to TBS positivity, approximately 11 to 11.5 days.

2.2. MC-003 ITV

MC-003 was a single-center, randomized placebo controlled, partially double-blind phase I clinical study of infection-treatment-vaccination (ITV) using chloroquine (CQ) with or without primaquine (PQ). There are six types of intervention. They are referred to as Arm 1a (PQd2/CQ/spz, n=2), Arm 1b (PQd2/CQ/spz, n=2), Arm2 (PQ/CQ/spz, n=11), Arm3 (PQ/CQ/no spz, n=5) serving as 'drug control', Arm4 (Cq/spz, n=3), and Arm5 (Infectivity Control, n=6). Detail of the study arm description can be found in the joint review (uni-review) by Drs. Shukal Bala, Rana Chattopadhyay, and Sue-Jane Wang.

- PQd3/CQ/spz (n=2) & PQd2/CQ/spz (n=2) – Arm 1

For analysis purpose, Arms 1a and 1b were combined. They were similar except the first PQ was administered on day 3 in PQd3/CQ/spz arm and on day 2 in PQd2/CQ/spz arm. It was of interest to also combine with Arm 2. However, there was one discordant pair in Arm 2 in that subject MC-003-012 receiving PQ/CQ/spz was TBS positive at day 11, but, was PCR negative. To avoid the contamination with the only discordant pair and given only 2 subjects were studied in Arm 1a and Arm 1b each, this reviewer evaluated Arms 1a and 1b separately from Arm 2. All subjects were found to be positive for malaria either by PCR or TBS.

Table 2.2.1 Time to positivity analysis censored at 28 days – MC-003 ITV Study

PQd3/CQ/spz (n=2) & PQd2/CQ/spz (n=2)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	11 (9, 11)	7 (na, na)	9 (7, 9)	10.5 (9, 11)
% censor	0%	0%	0%	0%
Nominal p-value	0.0014*			

*global test

Although only 4 subjects were studied, the gain in median time that facilitated early infection detection by PCR biomarker was approximately 2 days using at least 250 estimated parasites/mL criterion and approximately 4 days using any positive estimated parasites/mL criterion in subjects receiving either PQd3/CQ/spz or PQd2/CQ/spz in MC-003 ITV study, see Table 2.2.1. In this type of intervention, time to first clinical sign or symptom appeared to be similar to that with time to TBS positivity.

- PQ/CQ/spz (n=11) – Arm 2

With one false negative call with PCR assay, i.e., subject (b) (6) was TBS positive at day 11, but, was PCR negative, the ability of PCR assay to show a shorter time to PCR positivity than time to TBS positivity became less clear. In addition, subjects (b) (6) had missing rescue dates, missing TBS positivity date and missing PCR positivity date. These subjects were censored at day 28 in the time to positivity analysis.

Table 2.2.2.A Time to positivity analysis censored at 28 days – MC-003 ITV Study

PQ/CQ/spz (n=11)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	11 (9, 11)	7 (7, na)	9 (7, na)	11 (10, 12)
% censor	27%	36%	36%	9%
Nominal p-value	0.9399*			

*global test

Table 2.2.2.B Time to positivity analysis censored at days CHMI to rescue – MC-003 ITV Study

PQ/CQ/spz (n=11)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	11 (9, na)	7 (7, na)	9 (7, na)	11 (10, 12)
% censor	27%	36%	36%	9%
Nominal p-value	0.9228*			

*global test

Although the sample size in PQ/CQ/spz (Arm 2) is larger (n=11) compared to those types of intervention analyzed so far (n=4, 5, 6), the time to positivity analysis showed that there was no

difference among the four times when only one subject was a false negative PCR based on the statistical test, see Table 2.2.2.A or Table 2.2.2.B. It is interesting to note that there was still a numerically shorter median time to PCR positivity compared to time to TBS positivity, approximately 2 days shorter using at least 250 estimated parasites/mL criterion and approximately 4 days shorter using any PCR positivity criterion. The time to first clinical sign or symptom appeared to be similar to Time to TBS positivity.

- PQ/CQ/no spz (n=5) – Arm 3 (Drug Control)

Table 2.2.3 Time to positivity analysis censored at 28 days – MC-003 ITV Study

PQ/CQ/no spz (n=5)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	9 (9, 11)	7 (6, 7)	7 (7, 9)	10 (7, 12)
% censor	0%	0%	0%	0%
Nominal p-value	0.0019*			

*global test

For subjects receiving PQ/CQ/no spz in MC003 ITV study, there was no mosquito bites or DVI. It is interesting to note that time to any PCR positivity or time to at least 250 estimated parasites/mL gained approximately 2 days earlier than time to TBS positivity, see Table 2.2.3, though only 5 subjects were studied. The first time to clinical sign or symptom appeared to be approximately one day later than time to TBS positivity in this drug control arm. All subjects were found to be positive for malaria either by PCR or TBS.

- (CQ/spz) (n=3) – Arm 4

Table 2.2.4 Time to positivity analysis censored at 28 days – MC-003 ITV Study

CQ/spz (n=3)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	14 (13, 16)	10 (8, 10)	10 (10, 12)	10 (10, 13)
% censor	0%	0%	0%	0%
Nominal p-value	0.0219*			

*global test

Only 3 subjects receiving CQ/spz in MC-003 ITV study were assessed. There was a gain of approximately 4 days earlier with any PCR positivity criterion or at least 250 estimated parasites/mL criterion, see Table 2.2.4. In the CQ/spz intervention type, the time to first clinical sign or symptom appeared to be also approximately 4 days earlier than that with time to TBS positivity. All subjects were found to be positive for malaria either by PCR or TBS.

- Infectivity Control (n=6) – Arm 5

Table 2.2.5 Time to positivity analysis censored at 28 days – MC-003 ITV Study

n=6 infectivity control	Time to TBS +	Time to any	Time to ≥ 250	Time to 1st

		PCR+	estimated parasites/mL	clinical sign/symptom
Median (95% CI)	9 (7, 10)	7 (na, na)	7 (7, 8)	10 (8, 10)
% censor	0%	0%	0%	0%
Nominal p-value	< 0.0001*			

*global test

In MC003 ITV study, there were 6 infectivity control subjects. Table 2.2.5 showed that compared to median time to TBS positivity, approximately 2 days shorter to reach median time to any PCR positivity or median time to at least 250 estimated parasites/mL. However, the median time to first clinical sign or symptom appeared to be approximately 1 day later than that with time to TBS positivity. All subjects were found to be positive for malaria either by PCR or TBS.

2.3. PfSPZ-CVac PYR

This is a phase I single center study of ITV consisting of wild-type, aseptic, purified, cryopreserved *P. falciparum* sporozoites administered by DVI in conjunction with antimalarial chemoprophylaxis with CQ and pyrimethamine (PYR) to induce stage-specific sterile protection. The protocol-defined study arms were vaccinated three times. Arm 1a (n=2) and Arm 2 (n=9) included subjects receiving CQ and PYR. Arm 3 included those receiving CQ only (n=5). Arm 4 were the infectivity controls (n=5). The study was conducted in 2016 under IND 16650 at the NIH Clinical Center in Bethesda, Maryland, USA.

- PYR(D2,D3)+CQ (n=11)

Table 2.3.1.A Time to positivity analysis censored at 28 days – PfPZ-CVac PYR Study

PYR(D2,D3)+CQ (n=11)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	na (13, na)	8 (8, 15)	11 (11, 17)	14 (10, na)
% censor	64%	18%	18%	36%
Nominal p-value	0.0098*			

*global test

Table 2.3.1.B Time to positivity censored at days CHMI to rescue – PfPZ-CVac PYR Study

PYR(D2,D3)+CQ (n=11)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	18 (12, na)	8 (8, 15)	11 (11, 17)	14 (10, na)
% censor	64%	18%	18%	36%
Nominal p-value	0.0288*			

*global test

For subjects receiving PYR (D2,D3)+CQ intervention, due to relatively high censoring in time to TBS positivity, a more extreme censoring approach will push more subjects' censoring to 28 days in the time to TBS positivity than in other time to positivity. Such censoring pattern can

result in an optimistic nominal p-value of 0.0098 showing as if there is a highly significant finding in the difference in median time to positivity among the four groups.

Comparing between Table 2.3.1.A (more extreme censoring) and Table 2.3.1.B (more realistic censoring), the median time to TBS positivity can be calculated as18 days using a more realistic censoring approach. As shown in Table 2.3.1.B, a realistic censoring approach showed a gain in time to positivity with any PCR positivity criterion (approximately 10 days) or at least 250 estimated parasites/mL criterion (approximately 7 days). The time to first clinical sign or symptom was also shown to be approximately 4 days earlier than time to TBS positivity.

- CQ (n=5)

Table 2.3.2.A Time to positivity analysis censored at 28 days – PfPZ-CVac PYR Study

CQ (n=5)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	na (na, na)	na (9, na)	na (11, na)	na (na, na)
% censor	100%	80%	80%	80%
Nominal p-value	0.7795*			

*global test

Table 2.3.2.B Time to positivity censored at days CHMI to rescue – PfPZ-CVac PYR Study

CQ (n=5)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	na (na, na)	na (9, na)	na (11, na)	na (12, na)
% censor	100%	80%	80%	80%
Nominal p-value	0.7795*			

*global test

There were 5 subjects received CQ intervention. However, all 5 subjects were censored on TBS test and no median time to TBS positivity can be estimated. A high percentage of censoring was found in time to any PCR positivity (80%) or time to at least 250 estimated parasites/mL (80%). It was not possible to compare which assay will result in a shorter time to positivity as no median time had been reached yet, see Table 2.3.2.A and Table 2.3.2.B. Similar observation was made with high percentage of censoring in time to first clinical sign or symptom.

- Infectivity Control (n=5)

Table 2.3.3.A Time to positivity analysis censored at 28 days – PfPZ-CVac PYR Study

Infectivity Control (n=5)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	na (12, na)	7 (7, 9)	11 (8, na)	11 (7, na)
% censor	60%	0%	20%	20%
Nominal p-value	0.0019*			

*global test

Table 2.3.3.B Time to positivity censored at days CHMI to rescue – PfPZ-CVac PYR Study

Infectivity Control (n=5)	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	12 (12, na)	7 (7, 9)	11 (8, 11)	11 (7, na)
% censor	60%	0%	20%	20%
Nominal p-value	0.0023*			

*global test

Similar finding to MC-001 DEMO study and MC-003 ITV study, the infectivity control subjects in PfPZ-CVac PYR study showed a shorter median time to at least 250 estimated parasites/mL PCR positivity (approximately 1 day shorter) or any PCR positivity (approximately 5 days shorter). The median time to first clinical sign or symptom appeared to be 1 day shorter than that with time to TBS positivity, see Table 2.3.3.B (more realistic censoring) and Table 2.3.3.A (more extreme censoring).

3. Integrated assessment on time to positivity analysis

From the three clinical studies conducted, there were eight types of intervention. They are

- Infectivity Control
- CQ
- CQ/spz
- PQ/CQ/spz
- PQd2/CQ/spz
- PQD3/CQ/spz
- PQ/CQ/no-spz
- PYR(D2,D3)+CQ

There was an interest to assess the time to positivity analysis by pooling the infectivity control subjects from the three studies. From Section 2, we found that the gain in time to positivity with any PCR positivity criterion or at least 250 estimated parasites/mL criterion than with TBS positivity criterion was similarly observed in all three studies. Given there was no inconsistent finding among the three CHMI infectivity control subjects, this reviewer performed a meta-analysis by pooling data on CHMI infectivity control 'arm' or 'study', see Table 3A and Table 3B. This reviewer also evaluated time to positivity analysis for subjects whose intervention type is CQ, CQ/spz, PQ/CQ, or PQ/CQ/spz, see Table 4A and Table 4B.

- Infectivity Control across three studies

Table 3.A Time to positivity analysis censored at 28 days – infectivity controls across 3 studies

	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	11 (9, 12)	7 (na, na)	8 (7, 9)	10 (10, 12)
% censor	18%	0%	6%	6%
Nominal p-value	< 0.0001*			

*global test

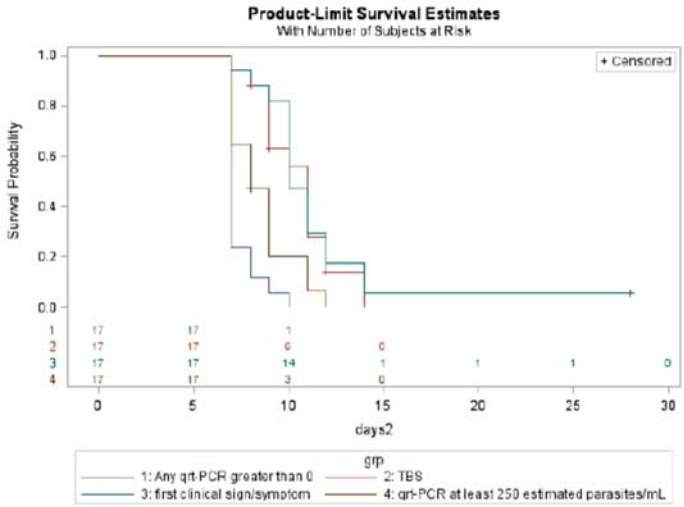
Table 3.B Time to positivity analysis censored at days CHMI to rescue treatment – infectivity controls across 3 studies

	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	11 (9, 12)	7 (na, na)	8 (7, 9)	10 (10, 12)
% censor	18%	0%	6%	6%
Nominal p-value	< 0.0001*			

*global test

By combining data on infectivity control subjects across the three studies, it seemed that approximately 3 days earlier with at least 250 estimated parasites/mL and approximately at least 4 days earlier with any PCR positivity were observed when they were compared to time to TBS positivity. The median time to first clinical sign or symptom was found to be approximately 1 day earlier than that with TBS positivity criterion, see Table 3.A and Table 3.B.

Figure 1. The Product-Limit Estimates on Time to Positivity by PCR Assay, TBS Assay and First Clinical Sign or Symptom – All Infectivity Control Subjects



To put in perspective of the results shown in Table 3.A and Table 3.B, this reviewer presents the product-limit survival estimates for the four curves: time to TBS positivity (red curve), time to any PCR positivity (blue curve), time to at least 250 estimated parasites/mL (brown curve), and time to first clinical sign or symptom (green curve) for visual comparison, see Figure 1.

From Figure 1, an accelerated time of infection detection based on PCR biomarker, either any PCR positivity or at least 250 estimated parasites/mL criteria, when compared to TBS positivity criterion is clearly shown with no crossing in curves. Figure 1A presents the negative log of the estimated time to positivity. The time to first clinical sign or symptom appears to be tracking the time to TBS positivity.

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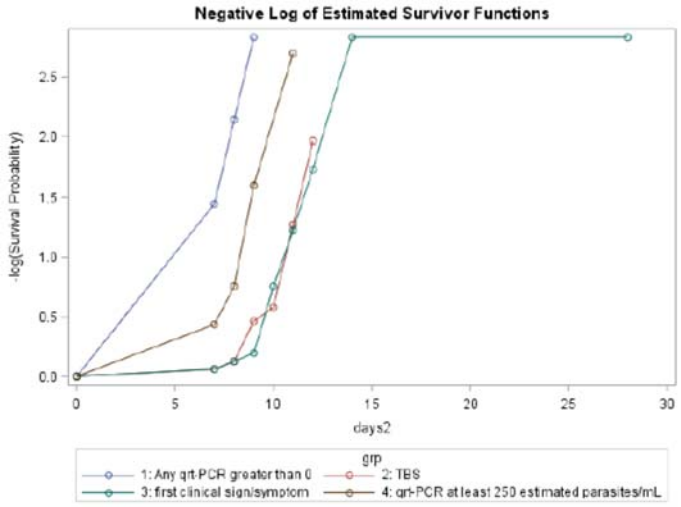
Figure 1A. The Product-Limit Estimates on Time to Positivity by PCR Assay, TBS Assay and First Clinical Sign or Symptom – All Infectivity Control Subjects



- Treated or Vaccinated Subjects from MC-003 study and PfSPZ-CVac study

Excluding infectivity control subjects and drug control subjects, Table 4.A and Table 4.B show the pooled time to positivity analysis in those subjects who received PQ or CQ with spz in MC-003 study and PfSPZ-CVac study.

Table 4.A Time to positivity analysis censored at 28 days – treated or vaccinated in MC-003 study and PfSPZ-CVac study

	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	15.5 (11, na)	8 (7, 11)	11 (9, 12)	11.5 (10, 14)
% censor	44%	29%	29%	26%
Nominal p-value	0.0374*			

*global test

Table 4.B Time to positivity analysis censored at days CHMI to rescue treatment – treated or vaccinated in MC-003 study and PfSPZ-CVac study

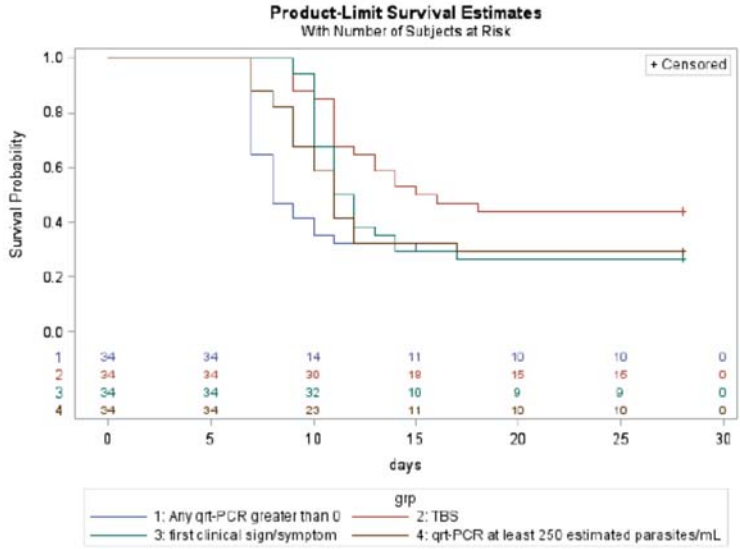
	Time to TBS +	Time to any PCR+	Time to ≥ 250 estimated parasites/mL	Time to 1st clinical sign/symptom
Median (95% CI)	14 (11, na)	8 (7, 11)	11 (9, 12)	11.5 (10, 14)
% censor	44%	29%	29%	26%
Nominal p-value	0.0558*			

*global test

It seemed that there was approximately 3 days earlier with at least 250 estimated parasites/mL and approximately at least 6 days earlier with any PCR positivity were observed when they were compared to time to TBS positivity, see Table 4.B. The median time to first clinical sign or symptom was found to be approximately 2.5 days earlier than that with TBS positivity criterion,

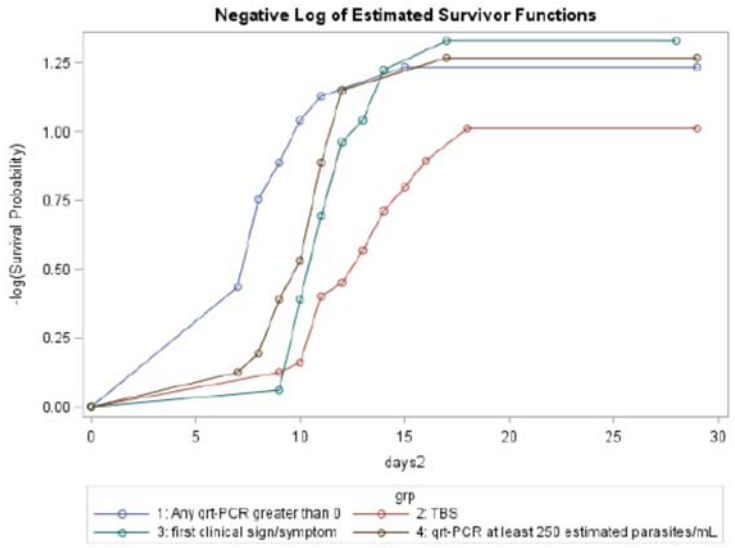
see Table 4.B, which may show 4 days earlier when the extreme censoring approach is adopted, see Table 4.A.

Figure 2. The Product-Limit Estimates on Time to Positivity by PCR Assay, TBS Assay and First Clinical Sign or Symptom – Treated or Vaccinated Subjects Excluding Drug Control Subjects



From Figure 2, the product-limit survival estimates for any PCR positivity or at least 250 estimated parasites/mL criterion showed shorter time than that with TBS positivity criterion. Similar trend is also observed from Figure 2A. There was also a numerically median shorter time in time to first clinical sign or symptom than in time to TBS positivity, see Figures 2 and 2A.

Figure 2A. The Product-Limit Estimates on Time to Positivity by PCR Assay, TBS Assay and First Clinical Sign or Symptom – Treated or Vaccinated Subjects Excluding Drug Control Subjects



V. Summary of Review Finding and Review Comments

Human infectivity controlled malaria infection subjects from Studies MC-001 DEMO, MC-003 ITV and PfSPZ-CVac were evaluated individually and across the studies. Comparing to the TBS criterion, the current gold standard, the results of this reviewer's time to positivity analyses consistently showed a shorter time to positivity with the proposed PCR biomarker using either any PCR positivity or at least 250 estimated parasites/mL criteria.

In subjects receiving drug or vaccine, depending on the type of intervention received, time to positivity analysis was performed by individual study and across the studies. Like what was observed in the infectivity control subjects, the proposed PCR biomarker consistently showed a shorter median time to positivity than TBS gold standard assay. There was a caveat. Of the three clinical studies, there was one false negative call with PCR assay in subject (b) (6) in MC-003 IVT study. The ability of PCR assay to show a shorter time to PCR positivity than time to TBS positivity became less clear statistically in MC-003 IVT study. However, the estimated median time to positivity with PCR appeared to be still shorter than that with TBS criterion. The false negative occurred approximately 2.5% to 3% in the treated or vaccinated subjects including versus excluding drug control subjects evaluated. When all treated or vaccinated subjects time to positivity were pooled, the overall median time to positivity was shorter with PCR biomarker than with TBS assay.

It is worth noting that in the drug control arm only studied in MC-003 ITV study, the median time to positivity with PCR biomarker was shorter by approximately 2 days than median time to TBS positivity. The median time to first clinical sign or symptom was approximately 1 day shorter than median time to TBS positivity.

It is noted that the sample size in the individual study were small. It is understood that the sample size is generally small for early phase drug or vaccine development. More data should be collected when the PCR biomarker is used as a tool for drug or vaccine development.

VI. Conclusion and Recommendation

The criterion of any PCR positivity and the criterion of at least 250 estimated parasites/mL were each shown to accelerate earlier detection of positivity for malaria. The data support the conclusion that PCR biomarker is superior than TBS assay and can be a replacement of gold standard TBS assay.

We recommend use of any PCR positivity as the criterion to monitor and inform initiation of rescue treatment with an anti-malarial drug at least 6 days following controlled human malaria infection (CHMI) with *P. falciparum* sporozoits in heathy subjects from non-endemic areas enrolled in clinical studies for vaccine and drug development against *P. falciparum*. The criteria have a better chance of early detection and are ethically sound given CHMI study enrolls healthy volunteers. The ability of earlier detection can also encourage normal healthy subjects to volunteer for CHMI study, which can accelerate drug or vaccine development in treating or preventing malaria.

References

Sauerwein RW, Roestenberg M, Moorthy VS. Experimental human challenge infections can acelerate clinical malaria vaccine development. Nature Review Immunology 2011;11(1):57-64.