Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

Summary Basis for Regulatory Action

Date:	March 18, 2024
From:	Miranda Oakley, Ph.D., Chair of the Review Committee
BLA/ STN#:	125808/0
Applicant Name:	Roche Molecular Systems, Inc US Highway 202 South Branchburg, NJ 08876 USA
Date of Submission:	May 24, 2023
MDUFA Goal Date:	March 22, 2024
Proprietary Name:	**cobas** Malaria

Established Name (common or usual name): The **cobas** Malaria test for use on the **cobas** 6800/8800 Systems

Intended Use/Indications for Use:
The **cobas** Malaria test for use on the **cobas** 6800/8800 Systems (**cobas** Malaria) is a qualitative in vitro nucleic acid screening test for the direct detection of *Plasmodium* (*P. falciparum, P. malariae*, *P. vivax, P. ovale* and *P. knowlesi*) DNA and RNA in whole blood samples from individual human donors, including donors of whole blood and blood components, as well as other living donors. It is also intended for use in testing whole blood samples to screen organ and tissue donors when samples are obtained while the donor's heart is still beating.

Whole blood samples from all donors are screened as individual samples.

The test is not intended for use as an aid in diagnosis of *Plasmodium* infection.
This test is not intended for use on samples of cord blood.
This test is not intended for use on cadaveric blood specimens.

Recommended Action: The Review Committee recommends licensure of this product.

Review Office Signatory Authority: Anne Eder, MD, PhD; Director, Office of Blood Research and Review

X I concur with the summary review.

☐ I concur with the summary review and include a separate review to add further analysis.

☐ I do not concur with the summary review and include a separate review.

Summary Basis for Regulatory Action

The table below indicates the material reviewed when developing the SBRA.

Table 1: Reviews Submitted

Document Title	Reviewer Name	Document Date
Product Review(s) (OBRR/DETTD) • *Clinical* • *Non-Clinical*	 Rob Duncan Caren Chancey Alain Debrabant Nitin Verma	 02/29/2024 02/23/2024 03/04/2024 03/06/2024
Statistical Review(s) • *Clinical and Non-Clinical (OBPV/DB/DNCE)*	Linye Song	11/28/2023
CMC Review • *CMC (OBRR/DETTD)* • *Facilities Review (OCBQ/DMPQ)* • *Microbiology Review (OCBQ/DBSQC)* • *Establishment Inspection Report(s) (OCBQ/DMPQ)*	 Brendan Elsworth Krishna (Mohan) Ketha Ma, Ou Yen Phan Ma, Ou	 03/05/2024 03/06/2024 03/04/2024 03/08/2024 03/04/2024
Labeling Review(s) • *OBRR/DETTD* • *APLB (OCBQ/APLB)*	 Miranda Oakley Sadhna Khatri	 03/08/2024 12/21/2023
Lot Release Protocols/Testing Plans/Testing Panel (OCBQ/DBSQC)	Matthew Arnold	03/11/2024
Bioresearch Monitoring Review (OCBQ/BIMO)	Solomon Murrell	03/14/2024
Software and Instrumentation (OBRR/DETTD)	Rana Nagarkatti	2/23/2024
Other living donor and cadaveric claim (OTP/DHT)	Hanh Khuu	2/21/2024

1. Introduction

The **cobas** Malaria test is manufactured at Roche Molecular Systems, Inc, U.S. Highway 202 South, Branchburg, NJ 08876, USA. This biologics license

application (BLA) for **cobas** Malaria test from Roche Molecular Systems, Inc. was received on May 24, 2023.

The application was assigned the number STN 125808/0 and granted a standard 10-month review status with a goal date of March 22, 2024. The application was filed June 18, 2023, and the mid-cycle meeting took place on October 30, 2023.

The BLA was preceded by pre-submission BQ200465/0 received on February 28, 2020 followed by one amendment (BQ200465/1) and focused on the regulatory aspects related to the design of pre-clinical (analytical) and clinical (sensitivity, specificity, and reproducibility) studies. Major issues also discussed include design of **cobas** primers and probes for the five *Plasmodium* species and inclusion of donor study population for clinical specificity studies. The **cobas** Malaria test is intended for use on the **cobas** 6800/8800 Systems. An investigational new drug application (IND) 28196 for this product was submitted on January 14, 2022, followed by six amendments; the last amendment was dated February 26, 2024.

Table 2: Chronological Summary of Submission and FDA Interaction with Roche Molecular Systems, Inc.

Date	Action	Amendment to BL125808
May 24, 2023	BLA CBER receipt	/0
June 1, 2023	Acknowledgement letter	
June 12, 2023	FDA IR- for Lot Release Protocol Template	
June 13, 2023	FDA IR- request for missing data in clinical specificity study	
June 14, 2023	Response to FDA IR dated June 12, 2023 received that contains RMS Lot Release Protocol Template	
June 20, 2023	Filing Notification Letter	
June 21, 2023	Response to FDA IR dated June 13, 2023 for clinical specificity studies	/0/1
June 26, 2023	FDA IR- request for "Omni Reagents and Common Components, Chemistry, Manufacturing, and Controls" and "References for Submission Structure" documents	
July 7, 2023	Response to FDA IR dated June 26, 2023	/0/2
August 21, 2023	FDA IR- for pre-clinical, clinical sensitivity, and clinical specificity studies	

August 28, 2023	Response to FDA IR dated August 21, 2023 for pre-clinical, clinical sensitivity, and clinical specificity studies	/0/3
October 25, 2023	FDA IR- for non-clinical and clinical studies and CMC issues	
October 27, 2023	Partial response to FDA IR dated October 25, 2023 received that addresses all non-clinical- and CMC-related questions	
October 30, 2023	FDA IR- for manufacturing and product quality related issues	
October 30, 2023	Information pertinent to FDA IR dated October 25, 2023	/0/4
October 31, 2023	FDA IR- for internal study related to protocol deviation in clinical study	
November 3, 2023	Complete response to FDA IR dated October 25, 2023 that addresses clinical study-related questions	/0/5
November 3, 2023	Response to FDA IR dated October 31, 2023 that contains study report	/0/6
November 14, 2023	FDA IR- containing various DMPQ-related issues	
November 14, 2023	Amendment received that contains documents updated and revised since initial submission	/0/7
November 21, 2023	FDA IR- for non-clinical and clinical studies and CMC issues	
November 22, 2023	Response to IR dated November 14, 2023	/0/8
November 29, 2023	RMS request for a meeting for FDA feedback related to FDA IR dated November 21, 2023 about the sensitivity of the pooled assay in the malaria endemic region study	
December 1, 2023	Points for discussion submitted by RMS for December 5, 2023 Teleconference	
December 5, 2023	Partial response to FDA IR dated November 21, 2023	/0/9
December 6, 2023	Teleconference between FDA and RMS to discuss sensitivity of assay for pooled samples	
December 8, 2023	Amendment received that contains minutes of December 6, 2023 teleconference to resolve issues related to pooling claim	/0/10

Summary Basis for Regulatory Action

January 4, 2024	FDA IR- address and contact information for shipment of coded panels	
January 4, 2024	FDA IR- related to product insert for the (b) (4) test	
January 5, 2024	Response to FDA IR dated January 4, 2024 containing shipment information	/0/11
January 18, 2024	Response to FDA IR dated January 4, 2024 related to product insert for (b) (4) test received	
January 19, 2024	Shipment of coded panels received by RMS for testing of (b) (4) lots of **cobas** Malaria against (b) (4) sets of coded panels	
January 19, 2024	Response to FDA IR dated January 4, 2024 and related to product insert	/0/12
January 24, 2024	FDA IR- related to Lot Release Protocol	
February 5, 2024	Response to FDA IR dated January 24, 2024	/0/13
February 23, 2024	FDA IR- for an updated Lot Release Protocol	
February 26, 2024	FDA IR – for status of blinded panels	
February 26, 2024	Response to FDA IR dated February 26, 2024 received stating that testing of blinded panels is ongoing	
February 26, 2024	FDA IR – for whole blood collection tube package insert	
February 27, 2024	Response to FDA IR dated February 26, 2024 containing whole blood collection tube package insert received	
February 27, 2024	RMS informs FDA that there is insufficient material in Set 1 Code 1 of the blinded malaria panel due to vial (b) (4)	
February 27, 2024	FDA IR – for red line version of whole blood collection tube package insert displaying differences in versions used for **cobas** Babesia versus **cobas** Malaria	
February 27, 2024	Amendment 14 received that contains whole blood collection tube package insert	/0/14
February 28, 2024	Response to FDA IR dated February 27, 2024 received	

February 29, 2024	Response to FDA IR dated June 12, 2023 that contains RMS Lot Release Protocol Template	/0/15
February 29, 2024	Response to FDA IR dated February 23, 2024 that contains updated Lot Release Protocol	/0/16
March 1, 2024	FDA IR – for reformatted repeatability data	
March 5, 2024	Response to FDA IR dated March 1, 2024	/0/17
March 6, 2024	Response to FDA IR dated February 26, 2024 that contains update on results of blinded panels	/0/18

2. Background

Malaria, a mosquito-borne disease, is caused by intraerythrocytic parasites belonging to the genus *Plasmodium*. Five *Plasmodium* species are known to cause disease in humans – *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. Among these, *P. falciparum* is the most transmitted and is responsible for fatalities around the world. In 2022, malaria was transmitted in 85 countries, causing approximately 249 million cases and 608,000 deaths. While malaria transmission is rare within the U.S., ten cases of locally-transmitted malaria were reported in four states in 2023. Approximately 2000 clinical cases are reported annually in the U.S., almost all due to infections acquired outside the country. Malaria can also be transmitted by transfusion of blood, blood products and solid organ transplantation collected from donors asymptomatically infected with *Plasmodium* parasites. In the U.S., about one case of transfusion-transmitted malaria (TTM) is reported every other year. Although TTM is rare, it can be fatal.

The **cobas** Malaria is a nucleic acid amplification test intended for the direct detection of *Plasmodium* (*P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi*) DNA and RNA in whole blood samples. The **cobas** Malaria test is configured in the same way as the licensed **cobas** Babesia test using the same negative control kit and the **cobas** 6800/8800 platform.

The **cobas** Malaria detects *Plasmodium* in a nucleic acid amplification assay that utilizes primers specific to the 18S rRNA gene. The assay is based on fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas** Malaria consists of the **cobas** Malaria test kit, the **cobas** Malaria control kit, the **cobas** NHP negative control kit, and the individual **cobas omni** reagents used for sample preparation.

Whole blood is collected in a designated Roche Whole Blood Collection Tube. Alternatively, whole blood may be collected in EDTA anticoagulant and transferred manually to the Roche Whole Blood Collection Tube. The Roche Whole Blood Collection Tube contains a pre-analytic, guanidine-based,

chaotropic reagent, used to lyse cells within the whole blood, releasing and preserving nucleic acids. The assay is designed to be performed on the **cobas** 6800/8800 Systems which consist of the sample supply module, transfer module, processing module, and analytic module. The tube containing lysed whole blood is loaded onto the **cobas** 6800/8800 Systems. Universal sample preparation, amplification of *Plasmodium* nucleic acid using primers unique to the 18S ribosomal gene, and detection of amplified product are then performed on this system. The assay utilizes an armored RNA internal control that serves as a full process control from sample preparation to amplification/detection and two external controls (positive and negative). Automated data management is executed by the **cobas** 6800/8800 Systems software, which assigns results for all tests as reactive, non-reactive, or invalid.

3. Chemistry Manufacturing and Controls (CMC)

The manufacture of the **cobas** Malaria is performed in accordance with Current Good Manufacturing Practices (cGMP) in an environmentally controlled facility.

a) Manufacturing Summary

The **cobas** Malaria is manufactured at Roche Molecular Systems, Inc. located at 1080 U.S. Highway 202 South, Branchburg, NJ 08876, USA (FDA Registration #: 2243471). The **cobas** 6800/8800 Systems are manufactured at Roche Diagnostics International, Ltd., (b) (4)

The **cobas** Malaria test (P/N 09352511190) consists of a 192-test cassette. The kit components are listed below:

- Proteinase solution (PASE): Nucleic acid extraction component.
- Internal control (IC): armored RNA internal control added to each sample.
- Elution Buffer (EB): Elution of purified nucleic acid.
- Master Mix Reagent 1 (MMX-R1): PCR buffer components.
- Master Mix Reagent 2 (MMX-R2): PCR buffer components, Primers, Probe, Aptamer and Enzymes.

The **cobas** Malaria Control Kit (P/N 09352520190), supplied separately, is used for quality control of **cobas** Malaria. The control kit consists of the following component:

- Malaria positive control: Armored *Plasmodium* RNA diluted in negative human plasma is used as a positive control for the **cobas** Malaria assay.

The **cobas** NHP Negative Control Kit (P/N 09051554190), supplied separately, is used for quality control of **cobas** Malaria. The control kit consists of the following component:

- Normal human Plasma (NHP): Human plasma that is free from *Plasmodium* nucleic acid is used as a negative control for the **cobas** Malaria assay.

Other general-purpose reagents and consumables for **cobas** 6800/8800 systems used for processing all **cobas** assays are listed below:

- Roche Whole Blood Collection Tube (P/N 08827907001): Whole blood to be tested is directly drawn into or added to this collection tube.
- **cobas omni** Processing Plate (P/N 05534917001): Reaction vessel.
- **cobas omni** Amplification Plate (P/N 05534941001): Reaction vessel.
- **cobas omni** Pipette Tips (P/N 05534925001): Disposable pipetting tips.
- **cobas omni** Liquid Waste Container (P/N 07094388001): Waste container.
- **cobas omni** Lysis Reagent (P/N 06997538190): Nucleic acid extraction component.
- **cobas omni** MGP Reagent (P/N 06997546190): Nucleic acid extraction component.
- **cobas omni** Specimen Diluent (P/N 06997511190): Nucleic acid extraction component.
- **cobas omni** Wash Reagent (P/N 06997503190): Nucleic acid extraction component.
- Solid Waste Bag (P/N 07435967001): Waste container.
- Solid Waste Container or Solid Waste Bag with Insert (P/N 07094361001 or 08030073001): Waste container.

b) Test Specifications

The analytical methods and their validations and/or qualifications reviewed for the **cobas** Malaria kit were found to be adequate for their intended use.

c) CBER Lot Release

The lot release protocol template was submitted to CBER for review and found to be acceptable after revisions. A lot release testing plan was developed by CBER and will be used for routine lot release.

d) Facilities Review/Inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facilities and inspectional history involved in the manufacturing of **cobas** Malaria assay are summarized in Table 3.

Table 3. Manufacturing facilities for cobas Malaria

Name/Address	FEI Number	DUNS Number	Inspection /Waiver	Justification/ Results
Roche Molecular Systems, Inc 1080 U.S. Highway 202 South Branchburg, New Jersey 08876	2243471	883238743	Waiver	ORA/OBPO December 2023; VAI

Manufacturing of all **cobas** Malaria kit components; **cobas** Malaria kit assembly, primary packaging, and labeling				

Acronym key: VAI– Voluntary Action Indicated; OBPO–Office of Biological Products Operations; ORA–Office of Regulatory Affairs

Inspection of the Roche Molecular System facility in Branchburg, New Jersey was waived. This facility was last inspected in December 2023 by ORA, and a Form FDA 483 with a list of observations was issued, classified as VAI. All inspectional issues have been resolved.

e) Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA has accepted this request because the manufacturing of this product will not significantly alter the concentration and distribution of naturally occurring substances. In addition, no extraordinary circumstances are known that would require an environmental assessment.

f) Container Closure

The assay components are packaged either in plastic tubes with plastic screw caps, or in plastic bottles with plastic plugs and induction seal. The system reagents are packaged in plastic bottles with plastic screw caps.

Container closure integrity is not assessed as all products are manufactured as bioburden controlled and contain preservatives.

4. Software and Instrumentation

The following is a summary overview of software, instrumentation and risk management information provided to support a reasonable assurance that the device is safe and effective for its intended uses and conditions of use.

a) Versioning

cobas 6800/8800 Systems Software version v1.4.7, **cobas** Malaria Assay Specific Analysis Package (ASAP) Software v12.2.0, **cobas** Synergy Software v1.4, **cobas** 6800/8800 Systems User Guide Publication version 5.1.

b) Device Description

The **cobas** Malaria test for use on the **cobas** 6800/8800 Systems (**cobas** Malaria) is a qualitative in vitro nucleic acid screening test for the direct detection of *Plasmodium* (*P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale* and *P. knowlesi*) DNA and RNA in individual whole blood samples. The system

consists of two separate instruments, the **cobas** 6800 System and the **cobas** 8800 Systems, which both provide fully integrated, automated sample preparation, nucleic acid extraction, and target amplification and detection.

Both systems can perform high throughput routine and priority testing while allowing continuous access and automated retesting. Positive sample ID is established and maintained with barcodes. Reagent cassettes (containing the test-specific reagents i.e., protease, internal control/quantitation standard, elution buffer, and master mix reagent) and control cassettes (containing the negative and positive controls) are stored on board at 2-8°C. Consumables are tracked for stability, availability, and expiration using barcodes and RFID tags. Each instrument consists of a sample supply module, a transfer module, a processing module, and an analytical module. The **cobas** 8800 instrument has an extra processing and analytical module resulting in a higher sample processing throughput, processing 960 samples in 8 hours, compared to the **cobas** 6800 instrument which can process 384 samples in 8 hours.

The main system functionality is provided by two software components: the **cobas** 6800/8800 Systems Software, which is the primary interface for operators to access, control and manage the system, and an ASAP, to implement the assay specific functionality. The **cobas** 6800/8800 Systems Software provides functionalities that are utilized by **cobas** Malaria and other Roche Molecular Systems (RMS) Blood Screening tests and includes, a Graphical User Interface (GUI), instrument and sample workflow management, database functionality, results reporting engine and integration with a Laboratory Information System (LIS) and to middleware, such as the **cobas** Synergy software. The **cobas** Synergy software is an optional software that can be used to receive orders and to transmit individual donor test results to the LIS.

The ASAP software includes parameters necessary for processing a sample on the instrument as well as parameters necessary for result calculation e.g., algorithms for raw signal processing, results calculation, pipetting parameters, configuration presentation, and associated metadata). The ASAP is built on a common framework for assays run on the **cobas** 6800/8800 instruments and is selectable on the system GUI to initiate testing and detection of specific analytes. Additional system functionalities and operation are described in the version-controlled user manual, and package inserts.

c) Risk Management

Risks related to donor test results, exposure of user to infectious disease agents, chemical, physical, and environmental hazards were evaluated. Major hazards include incorrect results, false positive and false negative donor test results, and moderate hazards include delayed results and physical hazards to user/operator. For the **cobas** Malaria test used on **cobas** 6800/8800 Systems, 91 risks were identified, including 41 for invalid results (delaying the availability of a rare blood group products or platelets to the recipient), 40 for false non-reactive results (resulting in release of contaminated blood/organ/tissue), eight

for false reactive results (resulting in a needlessly destroyed unit, rejection of an organ for transplantation or transient donor anxiety), and two for user infection. The final risk profile for the **cobas** Malaria test used on **cobas** 6800/8800 Systems after mitigations were implemented included 0 red risks, five yellow risks and 86 green risks. In addition, 28 risks associated with the Roche Whole Blood Collection Tubes (WBCT), are also applicable to the **cobas** Malaria assay. The final risk profile for the WBCT after mitigations were implemented included 0 red risks, four yellow risks and 24 green risks. The final risk profile for the **cobas** 6800/8800 Systems SW v1.4.7 after mitigations were implemented included 0 red risks, 29 yellow risks and 48 green risks. The final cybersecurity risk profile of the **cobas** 6800/8800 Systems SW and the **cobas** Malaria ASAP include 0 red risks, 16 yellow risks and eight green risks. Risk control measures were implemented to reduce all risks to acceptable levels.

The applicant stated that all risk control measures are implemented and verified, and that the labeling notifies the user of residual risks. Significant risk control measures include use of barcodes/RFID tags for sample and reagent tracking, automated checks for expiry of on board assay reagents and QC reagents, maintenance procedures, labeling and user manuals, database management activities, and access controls with individual usernames and passwords, firewalls and encryption, and configuration management, among others. The applicant concluded the overall residual risk of **cobas** 6800/8800 Systems is acceptable. This assessment appears to be supported by the evidence provided.

d) Unresolved Anomalies

The applicant indicated that no safety related anomalies were identified for the **cobas** Malaria ASAP v12.2.0. Unresolved anomaly information was provided and reviewed for the **cobas** 6800/8800 Systems and Software v1.4 in the **cobas** SARS-CoV-2 Qualitative 510 (k) (K213804), cleared on October 22, 2022. The risks associated with these unresolved anomalies were assessed to be acceptable.

e) Testing

Design verification was performed to confirm the design elements meet the specified requirements and includes verification of the effectiveness of risk control measures for potential causes of failure modes. This included software verification, software validation, testing at the unit level for each functionality and detailed integration testing for all functions and system level integration which was provided and reviewed for the **cobas** 6800/8800 Systems and Software v1.4 in the **cobas** SARS-CoV-2 Qualitative 510(k) (K213804), cleared on October 22, 2022. Test run results using the **cobas** Malaria test with donor samples were provided.

f) Development Management

The software development activities for each software component included establishing detailed software requirements, linking requirements with

associate verification tests, verification and validation, defects tracking, configuration management and maintenance activities to ensure the software conforms to user needs and intended uses.

5. Analytical Studies

Non-clinical studies were performed at Roche Molecular Systems, Inc, located at 1080 U.S. Highway 202 South, Branchburg, NJ 08876, USA. The analytical studies were conducted in compliance with 21 CFR Part 58 (Good Laboratory Practices or GLPs), as applicable.

a) Analytical Sensitivity

i) *Plasmodium falciparum* culture specimen diluted in whole blood (LoD)

The purpose of this study was to determine the Limit of Detection/Analytical Sensitivity of the **cobas** Malaria test when testing culture specimen of *P. falciparum* (*Pf*) diluted in whole blood (WB). Dilution series were prepared by (b) (4) Dilution series were prepared from 1.1 mL and (b) (4) input volumes in the Roche Whole Blood Collection Tube (WBCT); the 1.1 mL input volume represents the full draw volume, and the (b) (4) input volume represents a (b) (4) draw volume simulating sample collection at (b) (4). A guanidine-based additive (b) (4)) in the WBCT was used to lyse the cells within the whole blood, releasing and preserving nucleic acids. Each dilution series was tested using three reagent lots, with (b) (4) runs per day, on (b) (4) systems, over (b) (4) days, with a total of 45 replicates per level per dilution series per input volume. The resulting data was analyzed per input volume to determine the LoD based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate. The *Pf* LoD for 1.1 mL input was 2.9 iRBC/mL (95% CI 2.4-3.8) and the *Pf* LoD for (b) (4) input was (b) (4) iRBC/mL (95% CI (b) (4)

ii) LoD/Analytical Sensitivity of *P. falciparum* (Secondary Standard)

The purpose of this study was to determine the Limit of Detection/Analytical Sensitivity of the **cobas** Malaria test using an in-house Secondary Standard comprised of lysed *Pf* iRBCs (b) (4) iRBC/mL) prepared in (b) (4) at a (b) (4) ratio of WB:(b) (4). A dilution series of multiple replicates of this *Pf* Secondary Standard were prepared and tested. The LoD was determined based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate. The *Pf* LoD was 0.058 iRBC/mL (95% CI 0.049 – 0.071).

iii) LoD/Analytical Sensitivity of *P. knowlesi* (Secondary Standard)

The purpose of this study was to determine the Limit of Detection / Analytical Sensitivity of the **cobas** Malaria test when testing whole blood specimen containing *P. knowlesi* (*Pk*) target. Dilution series of the *Pk* in-house

Secondary Standard in WB:(b) (4) were prepared and tested in multiple replicates. This Roche in-house Secondary Standard contains *Pk* iRBCs in (b) (4) at a concentration of (b) (4) iRBC/mL The LoD was determined based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate. The *Pk* LoD was 0.044 iRBC/mL (95% CI 0.037 – 0.054).

iv) LoD/Analytical Sensitivity of *P. vivax* (Secondary Standard)

The purpose of this study was to determine the Limit of Detection/Analytical Sensitivity of the **cobas** Malaria test when testing a whole blood specimen containing *P. vivax* (*Pv*) target. Dilution series of the *Pv* Roche in-house Secondary Standard in WB:(b) (4) were prepared and tested in multiple replicates. This in-house Secondary Standard contains *Pv* iRBCs in (b) (4) at a concentration of (b) (4) iRBC/mL. The LoD was determined based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate. The *Pv* LoD was 0.012 iRBC/mL (95% CI 0.010 – 0.015).

v) Limit of Detection/Analytical Sensitivity of *Plasmodium* using Armored RNA in (b) (4) Specimen Diluent

The purpose of this study was to determine the Limit of Detection/Analytical Sensitivity of the **cobas** Malaria test when testing *Plasmodium* target using Armored RNA (aRNA) in generic specimen diluent. Armored RNA constructs, designed to represent the (b) (4) detection regions within the 18S gene (b) (4) , with sequences specific for each of the five *Plasmodium* species, were used to formulate the panels of testing material. When tested as specific pairs ("blend") of armored RNAs, the resulting amplification is specific for each *Plasmodium* species and inclusive of (b) (4) regions detected by the assay. Dilution series of aRNA blends for each *Plasmodium* species was prepared in (b) (4) Specimen Diluent and tested in multiple replicates. The LoD was determined based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate.

The *Pf* LoD (armored particles/mL) was 27.9 (95% CI 22.5 – 38.5). The *Pk* LoD (armored particles/mL) was 23.7 (95% CI 18.9 – 33.6). The *Pv* LoD (armored particles/mL) was 33.1 (95% CI 26.3 – 46.5). The *Pm* LoD (armored particles/mL) was 32.2 (95% CI 25.8 – 44.7. The *Po* LoD (armored particles/mL) was 59.0 (95% CI 44.1 – 90.3).

vi) Limit of Detection/Analytical Sensitivity of *Plasmodium* using Armored RNA (diluted in WB: (b) (4)

The purpose of this study was to determine the Limit of Detection/Analytical Sensitivity of the **cobas** Malaria test when testing whole blood specimen containing *Plasmodium* target using aRNA. Dilution series in WB:(b) (4) of aRNA blends for *Pf*, *Pm* and *Po* were prepared and tested in multiple replicates. The LoD was determined based on Probit Analysis and the lowest target concentration level with a ≥ 95% reactive rate.

The LoD of *Pf* (armored particles/mL) was (b) (4) (95% CI (b) (4)). The LoD of *Pm* (armored particles/mL) was (b) (4) (95% CI (b) (4) The LoD of *Po* (armored particles/mL) was (b) (4) (95% CI (b) (4)

Overall, the analytical sensitivity studies (studies i–vi) for the **cobas** Malaria were appropriately performed to evaluate the performance of the assay for all five *Plasmodium* species. The studies considered potential variables such as primer/probe performance and addressed limitations in availability of clinical specimens and parasite material produced in RBC cultures. For *P. falciparum*, the LoD was determined using intact iRBC, a secondary standard, and aRNA as detection targets. For *P. knowlesi* and *P. vivax*, the LoD was determined using secondary standards and aRNA as detection targets. For *P. malariae* and *P. ovale*, the LoD was determined using aRNA as a detection target due to lack of clinical specimens or cultured parasites. The data reviewed was found to be adequate and acceptable. The results are summarized in Table 4.

Table 4. Summary of cobas Malaria Limit of Detection (LoD)[1]

	LoD (spike iRBC)[2] iRBC/mL [95% CI] Dilution panel in WB (before lysis)	**LoD (2nd Std.)[3]** iRBC/mL (eq.) [95% CI] Dilution panel in WB:(b) (4)	**LoD (aRNA)** particles/mL [95% CI] Dilution panel in Buffer[4]	**LoD (aRNA)[5]** particles/mL [95% CI] Dilution panel in WB:(b) (4)
P. falciparum	**2.9** (1.1 mL input) [2.4 – 3.8] (b) (4) * input) (b) (4)	**0.058** [0.049–0.071]	**27.9** [22.5–38.5]	(b) (4)
P. knowlesi	na	**0.044** [0.037–0.054]	**23.7** [18.9–33.6]	na
P. vivax	na	**0.012** [0.010 – 0.015]	**33.1** [26.3–46.5]	na
P. malariae	na	na	**32.2** [25.8 – 44.7]	(b) (4)
P. ovale	na	na	**59.0** [44.1–90.3]	(b) (4)

na = not available; eq.=equivalent; WB= Whole Blood; (b) (4) = **cobas** (b) (4) (Guanidine) WB:(b) (4)

[1] Values represent 95% hit rate in Probit analysis

[2] Living synchronous ring stage *Pf* 3D7 strain

[3] Secondary Standards: *Pf*; *Pk*; *Pv*: (b) (4) iRBC/mL in WB:(b) (4)

[4] **cobas omni** specimen diluent
[5] aRNA= armored RNA encapsulated in MS2 bacteriophage
*Note: (b) (4) represents a (b) (4) draw volume simulating sample collection at (b) (4) .
All other studies in the table were done with an input sample volume of 1.1 mL

b) Cross reactivity/analytical specificity

The purpose of this study was to determine possible cross reactivity of the **cobas** Malaria test with microorganisms that could be present in clinical specimens. A total of 16 microorganisms (six different viruses, eight different bacteria, one parasite, and one yeast) (b) (4) whole blood: **cobas** PCR Media background (WB:(b) (4) in a ratio of (b) (4) were tested in (b) (4) in the presence of Malaria target (~3x LoD using *Pf* Secondary Standard) and in (b) (4) in the absence of Malaria target. The following microorganisms were evaluated: *Anaplasma phagocytophilum*, *Candida albicans*, *Parvovirus B19*, *Babesia microti*, *Chikungunya Virus*, *Staphylococcus aureus*, *Borrelia burgdorferi*, *Cutibacterium acnes*, *Staphylococcus epidermidis*, *Borrelia hermsii*, *Hepatitis B Virus*, *West Nile Virus*, *Borrelia parkerii*, *Hepatitis C Virus*, *Borrelia recurrentis*, *Human Immunodeficiency Virus*.

Non-reactive results were obtained with **cobas** Malaria for specimens spiked with the potentially cross-reacting microorganisms and not spiked with *Pf* Secondary Standard. Reactive results were obtained with **cobas** Malaria for specimens spiked with the potentially cross-reacting microorganisms and spiked with *Pf* Secondary Standard at ~3x LoD. All specimens had reactive (valid) internal control (IC) results. The results of the study demonstrate that there was no measurable cross-reactivity with the **cobas** Malaria assay from the potential cross-reactants tested as described above.

c) Inclusivity/Genotype Verification

The purpose of this study was to demonstrate consistent detection of all five *Plasmodium* species by **cobas** Malaria. Ten unique clinical specimens confirmed to be positive by microscopy for *P. falciparum* (*Pf*), *P. vivax* (*Pv*), *P. malariae* (*Pm*) and *P. ovale* (*Po*) were tested at two concentrations: neat and diluted to ~3x LoD. Due to clinical specimen unavailability of *P. knowlesi* (*Pk*), the Roche Secondary Standard for *Pk* was used for testing at ~3x LoD. *Plasmodium* positive clinical specimens were titer assigned using (b) (4) . All positive specimens for each species; *Pf*, *Pv*, *Pm* and *Po* were detected when tested neat and at ~3x LoD with the **cobas** Malaria test. All positive samples of *Pk* using the *Pk* Secondary Standard were also detected when tested at ~3x LoD (Table 5), demonstrating that **cobas** Malaria assay can detect all five *Plasmodium* species.

Table 5: Inclusivity/Genotype Verification of *Plasmodium* species

Species	Number of Clinical specimens	Tested Neat		Tested at 3x LoD	
		% Reactive	Ct Mean	% Reactive	Ct Mean
P. falciparum	10	100	(b) (4)	100	(b) (4)
P. vivax	10	100	(b) (4)	100	(b) (4)
P. knowlesi	0 [*Pk* Secondary Standard used]	na	(b) (4)	100	(b) (4)
P. malariae	10	100	(b) (4)	100	(b) (4)
P. ovale	10	100	(b) (4)	100	(b) (4)

na = not available
Ct = Cycle threshold

d) Dilutional Sensitivity

The purpose of this study was to evaluate the **cobas** Malaria test dilutional sensitivity for Malaria by testing serial dilutions of well characterized clinical specimens from (b) (4) unique donations from individuals infected with *P. falciparum* and from (b) (4) unique donations from individuals infected with *P. vivax*. Each specimen was diluted to (b) (4) LoD and (b) (4) LoD and tested with (b) (4) reagent lots for a total of (b) (4) replicates per concentration level. (b) (4) was used to titer assign the (b) (4) Malaria-positive clinical specimens. The data demonstrate an acceptable dilutional sensitivity for *P. falciparum* and *P. vivax* parasites at (b) (4) and (b) (4) LoD by **cobas** Malaria.

e) Sensitivity assessment using clinical specimens

The sensitivity of the **cobas** Malaria with clinical specimens was evaluated in-house using 100 clinical specimens confirmed by microscopy to be positive for *P. falciparum* or *P. vivax*. All specimens (61 *Pf* and 39 *Pv*) were pre-characterized, titer assigned, and titer verified using (b) (4). Each clinical specimen was diluted in WB:(b) (4) from a negative donor to the ~5x LoD and ~3x LoD and tested in multiple replicates. All 100 specimens had Malaria reactive results when tested at ~5x LoD and ~3x LoD (Table 6). For *P. falciparum*, clinical sensitivity was 100% (61/61) with a 95% confidence interval (CI) of 94.1% to 100%. Likewise, for *P. vivax*, clinical sensitivity was 100% (39/39) with a 95% CI of 91.0% to 100%.

Table 6: **Clinical Sensitivity for *P. falciparum* and *P. vivax***

Species	Dilution	Reactive Replicates/ Total Valid Replicates	Sensitivity (%) (95% confidence interval)
P. falciparum	~5x LoD	61/61	100% (94.1% – 100%)
P. falciparum	~3x LoD	61/61	100% (94.1% – 100%)
P. vivax	~5x LoD	39/39	100% (91.0% – 100%)
P. vivax	~3x LoD	39/39	100% (91.0% – 100%)

f) Specificity assessment using clinical specimens

The specificity of the **cobas** Malaria test with clinical specimens was evaluated in-house using 500 individual *Plasmodium*-negative whole blood specimens from healthy donors collected in a non-endemic region, determined negative by microscopy and stabilized in the Roche Whole Blood Collection Tube. All 500 specimens tested negative with the **cobas** Malaria demonstrating 100% specificity in a zero-prevalence population.

g) Endogenous Interference

The effect of potentially interfering endogenous substances on the **cobas** Malaria assay performance was evaluated by testing (b) (4) individual whole blood donor samples spiked with the potential interferents at the levels recommended by the CLSI guideline (b) (4) The potential interferents tested were triglycerides (33 g/L), hemoglobin (≥ 200 g/L), unconjugated bilirubin (684 µmol/L), albumin (60 g/L), and human DNA (2 mg/L). The individual donor samples spiked with *P. falciparum* Secondary Standard at ~3x LoD (no interferent) were used as Positive Spike Controls (PSC).

The results of the study demonstrated that all (b) (4) donor samples containing ~3x LoD *P. falciparum* target generated positive results indicating that the **cobas** Malaria test is not affected by the potential endogenous interfering substances tested. All target negative donor samples tested generated valid negative results for Malaria, indicating that specificity of **cobas** Malaria was not affected by the potential endogenous interfering substances tested.

h) Exogenous Interference

The purpose of this study was to evaluate the influence of potentially interfering exogenous substances on the performance of the **cobas** Malaria test. The effect of potential interferents was evaluated by testing whole blood samples from (b) (4) individual donors spiked *P. falciparum* Secondary Standard at ~3x LoD and with the potential interferents at levels recommended in CLSI (b) (4) and (b) (4). The following potential interferents were tested: acetaminophen, acetylsalicylic acid, atorvastatin, atovaquone, azithromycin, fluoxetine, loratadine, atenolol, naproxen, paroxetine, sertraline, ascorbic acid, ibuprofen, and phenylephrine HCl. The individual donor samples spiked only with *P. falciparum* Secondary Standard at ~3x LoD (no interferent) were used as PSC.

All target negative donor samples spiked with interferent generated valid negative results indicating that the specificity of **cobas** Malaria was not affected by the potential exogenous interferents tested. All donor samples containing ~3x LoD Malaria target and spiked with interferent generated positive results indicating that sensitivity of the **cobas** Malaria test is not affected by the potential exogenous interferents tested.

i) Precision

The purpose of this study was to evaluate the precision/repeatability of the **cobas** Malaria for: *P. falciparum*, *P. knowlesi*, and *P. vivax*. This study was performed using (b) (4) different lots of **cobas** Malaria test kits and **cobas** Malaria control kits, on (b) (4) different instruments, across (b) (4) different days, by (b) (4) different operators (dilution series), with (b) (4) runs per day. Results for precision were assessed for each *Plasmodium* species. Each panel consisted of the Roche in-house Secondary Standard (b) (4) iRBC/mL) diluted in WB:(b) (4) at ~2.5x, 1.0x and 0.5x LoD. Additional sources of variability, e.g., lot to lot, operator to operator, were evaluated in the Reproducibility study.

Precision data for positive panel members with reactive **cobas** Malaria by *Plasmodium* species and expected *Plasmodium* concentration demonstrated limited overall variability for the Cycle threshold (Ct) values for the ~0.5x LoD, ~1x LoD, and ~2.5x LoD panel members of each *Plasmodium* species. The highest within-laboratory % CV observed was 3.0% for *P. vivax* at ~0.5x LoD and the lowest within-laboratory % CV was 1.6% for *P. falciparum* at ~2.5x LoD. For all other positive panel members from all species, the within-laboratory % CV were ≤ 2.8%. The within-laboratory % CV decreased for each *Plasmodium* species, as the target parasite concentration increased from ~0.5x LoD to ~2.5x LoD. The results are summarized in Table 7.

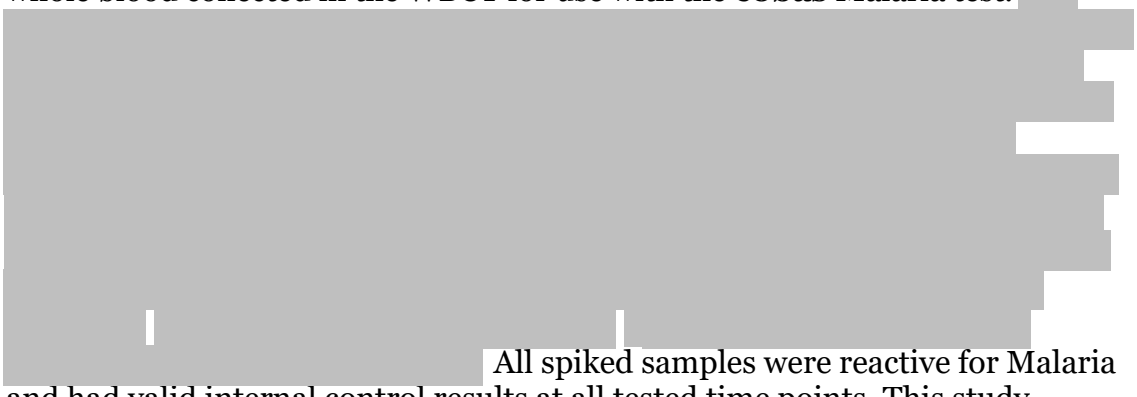
Table 7. Within Laboratory Precision (Repeatability) by Malaria Species

Malaria Species	Expected *Plasmodium* Con.	n*/N	Mean Ct	Repeat-ability SD	Repeat-ability % CV	Within-laboratory SD	Within-laboratory % CV
Pf	0.026iRBC/mL (~0.5x LoD)	151/198	37.4	0.84	2.3%	0.90	2.4%
Pf	0.052iRBC/mL (~1x LoD)	187/198	36.8	0.76	2.1%	0.81	2.2%
Pf	0.13 iRBC/mL (~2.5x LoD)	197/197	35.9	0.56	1.6%	0.59	1.6%
Pk	0.020 iRBC/mL (~0.5x LoD)	153/198	37.1	0.98	2.6%	1.03	2.8%
Pk	0.039 iRBC/mL (~1x LoD)	186/198	36.6	0.82	2.3%	0.83	2.3%
Pk	0.078 iRBC/mL (~2.5x LoD)	196/198	36.0	0.65	1.8%	0.73	2.0%
Pv	0.007 iRBC/mL (~0.5x LoD)	158/198	37.2	1.05	2.8%	1.13	3.0%
Pv	0.013 iRBC/mL (~1x LoD)	194/198	36.6	0.77	2.1%	0.78	2.1%
Pv	0.033 iRBC/mL (~2.5x LoD)	198/198	35.5	0.70	2.0%	0.80	2.2%

Con. = concentration, SD = Standard Deviation, % CV = percent coefficient of variation, iRBC = infected red blood cells, LoD = limit of detection, *Pf = Plasmodium falciparum*, *Pk = Plasmodium knowlesi, Pv = Plasmodium vivax.*
*n is the number of reactive tests that contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

j) Whole Blood collected in Roche WBCT

The purpose of this study was to determine the clinical specimen stability of whole blood collected in the WBCT for use with the **cobas** Malaria test. (b) (4)



All spiked samples were reactive for Malaria and had valid internal control results at all tested time points. This study demonstrated that WB collected in the Roche WBCT can be stored for up to 60 days at 2–8°C, including 24 hours at up to 30°C or 72 hours at up to 25°C. Samples can also be stored for up to 12 months at –20°C, including a period of 24 hours at up to 30°C, 72 hours at up to 25°C or 12 days at up to 2–8°C. Frozen samples can be subjected to three freeze/thaw cycles.

k) Whole Blood collected in EDTA

The purpose of this study was to determine the clinical specimen stability of whole blood collected in EDTA for use with the **cobas** Malaria test. The sample preparation was done as above for the Roche WBCT except that the WB from (b) (4) donors were collected in EDTA tubes. Testing was performed at (b) (4) time points while samples were stored at different temperatures and lengths of times, before or after transfer to the Roche WBCT. Prior to testing, (b) (4) of the spiked (b) (4) iRBC/mL) EDTA whole blood was transferred to a Roche WBCT. All timepoints had acceptable results, except (b) (4) (Spiked EDTA WB stored 24 hours at 30°C + 48 hours at 25°C + 10 days at 2-8°C prior to dilution in Roche WBCT), which generated 90% (b) (4) replicates) reactivity with the **cobas** Malaria assay. Additional replicates were prepared from the original (b) (4) non-reactive EDTA whole blood samples and were tested in (b) (4) using new stabilized WBCTs and were Malaria reactive. Furthermore, results of (b) (4) subsequent timepoints (b) (4) storage conditions plus additional storage at 25°C for 36 hours or 48 hours after dilution in Roche WBCT) were found to be acceptable. This study demonstrated that WB collected in EDTA can be stored for up to 12 days at 2–8°C, including a period of 24 hours at up to 30°C or 72 hours at up to 25°C. After dilution in the Roche WBCT, the sample may be stored for up to 48 hours up to 25°C. Under freezing conditions, samples were stable for up to 30 days including three freeze/thaw cycles after transfer in the Roche WBCT.

l) Kit Lot Interchangeability

The purpose of this study was to determine the interchangeability of lots for the **cobas** Malaria test when testing (b) (4) lot combinations of reagents following established kit cassette and Roche Manufactured Controls (RMC) release procedures. (b) (4) unique reagent kit lot combinations were tested using the release test procedure of test-specific reagent cassette for the **cobas** Malaria test.

The **cobas** Malaria Kit Lot Interchangeability study demonstrates that there are no lot-to-lot dependencies within the **cobas** Malaria test kits. (b) (4) out of (b) (4) lot combinations generated results within the release test procedure specifications. The results of the study indicate that different lots of test specific reagents, **cobas omni** reagents and RMC can be used interchangeably.

m) Whole System Failure (Robustness)

The objective of this Technical Performance Verification (TPV) study was to determine the rate of the Whole System Failure/Robustness (meaning the overall rate of failures that lead to false negative results) for **cobas** Malaria. *P. falciparum* Secondary Standard (b) (4) iRBC/mL) was used to generate the panel in WB:(b) (4) at approximately 3x LoD. A total of 100 replicates of the ~3x LoD panel were tested. The acceptance criteria were that the Whole System Failure Rate must be (b) (4). The results had a 0% failure rate (95% CI 0–3.6%) indicating the robustness of the system.

n) IC Failure Rate, Control Failure Rate and Sample Reliability

The objective of this analysis was to evaluate the Failure Rates of the Internal Control (IC) and RMC of the **cobas** Malaria test using data from (b) (4) technical performance verification studies. The acceptance criteria for this study were that the IC failure rate for negative and positive samples combined must be (b) (4), the control failure rate must be (b) (4), and sample reliability shall be at least (b) (4) for whole blood specimens.

For RMC Failure Rate and Sample Reliability, the results meet the acceptance criteria. For IC Failure Rate, combined results from TPV studies and Specificity clinical trial data does not meet the acceptance criteria of IC Failure Rate at (b) (4).

o) On Board and Open Kit Stability (including reusability)

The objective of this study was to determine the on board and Open Kit Stability of the **cobas** Malaria Reagent kits at (b) (4) time points over the course of (b) (4) days (b) (4) The opened reagent kits were tested for a total of (b) (4) Reusability was also assessed by using the reagent kit 40 times (mock runs) followed by a functional release test. (b) (4) reagent kits were included in the on board stability study and (b) (4) kits were used in the reusability study. Data from the (b) (4) runs was included for analysis.

The data support the reagent expiry conditions claimed in the package insert. The **cobas** Malaria test specific reagent kit 192T has an Open Kit stability of 90 days and an on board stability of 40 hours at (b) (4) when used on the cobas 6800/8800 systems. The 192T test-specific reagent kits can be re-used 40 times.

p) RMC On Board Stability

The purpose of this study was to evaluate the on board stability of the RMC for the **cobas** Malaria test for use on the **cobas** 6800/8800 Systems. (b) (4) vials of **cobas** Malaria RMC were (b) (4) (simulating the on board temperature) followed by storage at 2-8°C. Testing of the RMC on board stability was conducted according to the RMC release procedures. In addition to the (b) (4) RMC vials, (b) (4) vials of (b) (4) RMC were tested as reference material in the same run. Testing was performed using (b) (4) of **cobas** Malaria test kit and (b) (4) **cobas** 8800 System. The results demonstrate that the **cobas** Malaria RMC is stable for (b) (4) and support an on board instrument stability claim of 10 hours.

q) Specimen Type Matrix Equivalency

The purpose of this study was to demonstrate equivalence of whole blood donor samples collected in EDTA tubes versus samples collected directly into the Whole Blood Collection Tube. (b) (4) replicates of each specimen type were tested with and without *Plasmodium falciparum* spiked at (b) (4) LoD for each donor (b) (4) different donors). Testing was performed using (b) (4) of **cobas**

Malaria test kit and (b) (4) **cobas** 8800 systems, over the course of (b) (4) days. Both specimen types (whole blood donor samples collected in EDTA tubes and whole blood donor samples collected directly into the Whole Blood Collection Tube) resulted in 100% reactivity (b) (4) for the spiked Malaria condition and 100% negativity (b) (4) for the un-spiked Malaria condition.

6. Clinical Studies

Clinical studies were performed to evaluate the clinical specificity, sensitivity, and reproducibility of the **cobas** Malaria test. Clinical specificity was evaluated in a U.S. population who had no known risk of malaria exposure as well as a small sample size of donors who were deferred for malaria-risk. Clinical sensitivity was evaluated in known *Plasmodium*-positive samples and malaria asymptomatic individuals living in an endemic area of Nigeria. The reproducibility of **cobas** Malaria was evaluated across lot, site/system, day, and batch and within-batch.

a) Clinical Specificity

The clinical specificity of **cobas** Malaria was evaluated on 20,187 individual blood donations collected at three different test sites (21.22% (4,284) from the American Red Cross, 47.24% (9,536) from Bloodworks Northwest, and 31.54% (6,367) from Gulf Coast Regional Blood Center). All 20,187 donations tested individually on **cobas** Malaria were non-reactive, demonstrating 100% clinical specificity (95% exact CI: 99.98%, 100.00%). No follow up testing was performed on **cobas** Malaria non-reactive donations in this study.

Table 8. Comparison of cobas Malaria with donation status

	Donation Status* Positive n (%)	**Donation status negative n (%)**	**Donation status unresolved n (%)**	**Total N**
Reactive	0 (0.000)	0 (0.000)	0 (0.000)	0
Non-reactive	0 (0.000)	20,187 (100.000)	0 (0.000)	20, 187
Total	0	20,187	0	20, 187

* Donation Status was assigned based on the testing reactivity pattern observed on the index donation (initial and additional index testing) and/or based on follow-up study results.

b) Deferred Donors

An additional study was performed on 159 samples from donors from each study site who were deferred due to the site-specific donor intake questionnaire responses about travel to or residence in malaria-endemic areas. One hundred percent of these samples were non-reactive for **cobas** Malaria. These results suggest that no *Plasmodium* infections were detected by the **cobas** Malaria test in a small population of malaria-risk donors in this study.

Table 9. Comparison of cobas Malaria with donation status-deferred donors

	Donation Status* Positive n (%)	Donation status negative n (%)	Donation status unresolved n (%)	Total N
Reactive	0 (0.000)	0 (0.000)	0 (0.000)	0
Non-reactive	0 (0.000)	159 (100.000)	0 (0.000)	159
Total	0	159	0	159

* Donation Status was assigned based on the testing reactivity pattern observed on the index donation (initial and additional index testing) and/or based on follow-up study results.

c) Malaria-Endemic Region Study

A total of 199 samples collected in August-September 2021 from asymptomatic subjects in an endemic region in Nigeria were tested individually by **cobas** Malaria and an alternate nucleic acid test (ALT NAT) assay which uses primers and probes that are different from those used in the **cobas** Malaria. Results demonstrated that 77/199 (38.7%) were **cobas** reactive and 122/199 (61.3%) were **cobas** non-reactive. Additionally, 83/199 (41.7%) were ALT NAT positive and 116/199 (58.3%) were ALT NAT negative in the study (Table 8). The positive percent agreement between the **cobas** Malaria and ALT NAT was 91.6%. There were eight samples that had discordant results between **cobas** Malaria and ALT NAT; seven were **cobas** Malaria non-reactive and ALT NAT positive and one was **cobas** Malaria reactive and ALT NAT Negative. The true status of these eight discordant samples could not be established because a third confirmatory assay was not performed on these samples.

Table 10. Results of Cobas Malaria and ALT NAT in a malaria endemic region study

cobas Malaria Result	ALT NAT Positive	ALT NAT Negative	Total
Reactive	76	1	77
Non-Reactive	7	115	122
Total	83	116	199
PPA (95% Exact CI)	91.6% (83.6%, 95.9%)	-	-
NPA (95% Exact CI)	99.1% (95.3%, 99.8%)	-	-
OPA (95% Exact CI)	96.0% (92.3%, 97.9%)	-	-

CI: confidence interval; NAT: nucleic acid test; NPA: negative percent agreement, OPA: overall percent agreement; PPA: positive percent agreement.

d) Clinical Sensitivity

The clinical sensitivity of **cobas** Malaria was evaluated in a study testing 417 known *Plasmodium*-positive samples. These consisted of 237 clinical samples (118 *P. falciparum*, 118 *P. vivax*, and 1 *P. malariae*) and 180 contrived samples (36 spike samples:12 each at low, medium, and high concentration for each of the five *Plasmodium* species, *falciparum*, *vivax*, *ovale*, *knowlesi*, and *malariae*). All of the 417 neat samples were reactive with **cobas** Malaria resulting in a clinical sensitivity of 100% for overall (clinical and contrived combined) neat known *Plasmodium*-positive samples. The corresponding two-sided 95% exact CI for sensitivity was (99.1%, 100%).

Table 11. Clinical Sensitivity- positive neat samples

Dilution	Sample Type	Species	Total Known *Plasmodium*-Positive Samples	Number Reactive	Sensitivity Estimate	95% Exact CI
Neat	Overall	n/a	417	417	100.0%	(99.1%, 100.0%)
Neat	Clinical/Contrived	*P. falciparum*	154	154	100.0%	(97.6%, 100.0%)
Neat	Clinical/Contrived	*P. malariae*	37	37	100.0%	(90.5%, 100.0%)
Neat	Clinical/Contrived	*P. vivax*	154	154	100.0%	(97.6%, 100.0%)
Neat	Contrived	*P. ovale*	36	36	100.0%	(90.3%, 100.0%)
Neat	Contrived	*P. knowlesi*	36	36	100.0%	(90.3%, 100.0%)

e) Reproducibility

The purpose of this study was to evaluate the reproducibility of **cobas** Malaria across lot, site/system, day, batch, and within-batch. Reproducibility was evaluated across the following factors at three sites (two external and one internal): lot (three manufactured lots of reagent), site/system (three test sites: at least one test site had a **cobas** 6800 system and at least one test site had a **cobas** 8800 system), day (five days of testing per lot), batch (two valid batches per day: 1 batch=1 panel + 2 controls), and within-batch (three replicates of each concentration per batch). The total number of test results per concentration were 3 lots x 3 sites x 5 days x 2 batches x 3 replicates/concentration=270 test results/concentration. The test panel was comprised of samples of *P. falciparum*, *P. malariae*, *P. ovale* and *P. knowlesi* positive blood each at three concentrations (~0.5x LoD, ~1-2x LoD and ~3x LoD).

The mean, SD, and CV (%) of Ct values for positive panel members that are **cobas** Malaria reactive and for within-batch and between batch (Table 12) and day, site, and lot (Table 13) by expected *Plasmodium* concentration and *Plasmodium* species are presented. The total SD and total CV (%) for all variance components are presented in Table 14. Only a small overall variability

was observed for the Ct values across site, lot, day, or batch or within-batch for *Plasmodium* panel members at ~0.5x LoD, ~1-2x LoD, or ~3x LoD. The highest total CV (%) observed was 2.5% for *P. vivax* at ~0.5× LoD. The lowest total CV (%) observed was 1.4% for *P. ovale* at ~3× LoD. The total CV (%) for all other positive panel members from all species were ≤ 2.3%. Within each species, the total CV (%) decreased as the target parasite concentration increased from ~0.5× LoD to ~3× LoD. The data presented demonstrate acceptable reproducibility.

Table 12: Within-Batch and Between-Batch Standard Deviations and Coefficients of Variation (%) for Cycle Threshold by *Plasmodium* Species and Expected *Plasmodium* Concentration (Positive Panel Members)

Malaria Species	Expected Con.	n*/N	Mean Ct	Within-Batch SD	Within-Batch CV%	Between-Batch SD	Between-Batch CV%
Pf	~0.5 x LoD	229/270	37.1	0.73	2.0%	0.00	0.0%
Pf	1-2 x LoD	270/270	36.1	0.72	2.0%	0.00	0.0%
Pf	~3 x LoD	270/270	35.4	0.59	1.7%	0.21	0.6%
Pv	~0.5 x LoD	251/270	37.0	0.88	2.4%	0.09	0.3%
Pv	1-2 x LoD	269/269	35.8	0.70	1.9%	0.08	0.2%
Pv	~3 x LoD	269/269	35.0	0.64	1.8%	0.00	0.0%
Po	~0.5 x LoD	211/270	37.3	0.75	2.0%	0.00	0.0%
Po	1-2 x LoD	269/270	36.4	0.68	1.9%	0.10	0.3%
Po	~3 x LoD	270/270	35.5	0.48	1.4%	0.00	0.0%
Pm	~0.5 x LoD	210/270	37.6	0.79	2.1%	0.00	0.0%
Pm	1-2 x LoD	263/269	36.9	0.59	1.6%	0.14	0.4%
Pm	~3 x LoD	270/270	36.1	0.48	1.3%	0.16	0.4%
Pk	~0.5 x LoD	220/270	37.3	0.81	2.2%	0.27	0.7%
Pk	1-2 x LoD	270/270	36.3	0.71	2.0%	0.00	0.0%
Pk	~3 x LoD	270/270	35.5	0.62	1.8%	0.00	0.0%

Note: SD = standard deviation, CV(%) = percent coefficient of variation, Ct = cycle threshold, LoD = limit of detection, Con. = concentration, *Pf* = *Plasmodium*

falciparum, *Pv = Plasmodium vivax*, *Po = Plasmodium ovale*, *Pm = Plasmodium malariae*, *Pk = Plasmodium knowlesi*.

* n is the number of reactive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Table 13. Day, Site, and Lot Standard Deviations and Coefficients of Variation (%) for Cycle Threshold by *Plasmodium* Species and Expected *Plasmodium* Concentration (Positive Panel Members)

Malaria Species	Expected Con.	n*/N	Mean Ct	Day SD	Day CV%	Site SD	Site CV%	Lot SD	Lot CV%
Pf	~0.5 x LoD	229/270	37.1	0.25	0.7%	0.00	0.0%	0.10	0.3%
Pf	1-2 x LoD	270/270	36.1	0.07	0.2%	0.00	0.0%	0.15	0.4%
Pf	~3 x LoD	270/270	35.4	0.00	0.0%	0.07	0.2%	0.05	0.1%
Pv	~0.5 x LoD	251/270	37.0	0.18	0.5%	0.00	0.0%	0.16	0.4%
Pv	1-2 x LoD	269/269	35.8	0.00	0.0%	0.01	0.0%	0.00	0.0%
Pv	~3 x LoD	269/269	35.0	0.13	0.4%	0.11	0.3%	0.07	0.2%
Po	~0.5 x LoD	211/270	37.3	0.00	0.0%	0.00	0.0%	0.00	0.0%
Po	1-2 x LoD	269/270	36.4	0.00	0.0%	0.06	0.2%	0.00	0.0%
Po	~3 x LoD	270/270	35.5	0.00	0.0%	0.10	0.3%	0.00	0.0%
Pm	~0.5 x LoD	210/270	37.6	0.00	0.0%	0.17	0.5%	0.09	0.2%
Pm	1-2 x LoD	263/269	36.9	0.00	0.0%	0.03	0.1%	0.18	0.5%
Pm	~3 x LoD	270/270	36.1	0.06	0.2%	0.15	0.4%	0.12	0.3%
Pk	~0.5 x LoD	220/270	37.3	0.00	0.0%	0.07	0.2%	0.09	0.2%
Pk	1-2 x LoD	270/270	36.3	0.00	0.0%	0.10	0.3%	0.13	0.3%
Pk	~3 x LoD	270/270	35.5	0.10	0.3%	0.08	0.2%	0.00	0.0%

Note: SD = standard deviation, CV(%) = percent coefficient of variation, Ct = cycle threshold, LoD = limit of detection, Con. = concentration, *Pf = Plasmodium falciparum*, *Pv = Plasmodium vivax*, *Po = Plasmodium ovale*, *Pm = Plasmodium malariae*, *Pk = Plasmodium knowlesi*

* n is the number of reactive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

Table 14: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold by *Plasmodium* Species and Expected *Plasmodium* Concentration (Positive Panel Members)

Species	Expected Con.	n*/N	Mean Ct	Total SD	Total CV%
Pf	~0.5 x LoD	229/270	37.1	0.78	2.1%
Pf	1-2 x LoD	270/270	36.1	0.73	2.0%
Pf	~3 x LoD	270/270	35.4	0.64	1.8%
Pv	~0.5 x LoD	251/270	37.0	0.92	2.5%
Pv	1-2 x LoD	269/269	35.8	0.70	2.0%
Pv	~3 x LoD	269/269	35.0	0.66	1.9%
Po	~0.5 x LoD	211/270	37.3	0.75	2.0%
Po.	1-2 x LoD	269/270	36.4	0.69	1.9%
Po	~3 x LoD	270/270	35.5	0.49	1.4%
Pm	~0.5 x LoD	210/270	37.6	0.81	2.2%
Pm	1-2 x LoD	263/269	36.9	0.64	1.7%
Pm	~3 x LoD	270/270	36.1	0.54	1.5%
Pk	~0.5 x LoD	220/270	37.3	0.86	2.3%
Pk	1-2 x LoD	270/270	36.3	0.73	2.0%
Pk	~3 x LoD	270/270	35.5	0.64	1.8%

Note: SD = standard deviation, CV(%) = percent coefficient of variation, Ct = cycle threshold, LoD = limit of detection, Con. = concentration, *Pf.* = *Plasmodium falciparum*, *Pv.* = *Plasmodium vivax*, *Po.* = *Plasmodium ovale*, *Pm.* = *Plasmodium malariae*, *Pk* = *Plasmodium knowlesi*

* n is the number of reactive tests, which contribute Ct values to the analysis. N is the total number of valid tests for the panel member.

7. BIMO – Clinical/Statistical/Pharmacovigilance

Bioresearch Monitoring (BIMO) Clinical Investigator inspections were issued for three clinical study sites that participated in the conduct of study protocol cX8-MAL-521 and cX8-MAL-520. Two of three inspections are complete, and a review

of the inspection reports did not reveal issues that impact the data submitted in this Biologics License Application.

8. Pediatrics

N/A

9. Other Special Populations

N/A

10. Advisory Committee Meeting

N/A

11. Other Relevant Regulatory Issues

N/A

12. Labeling

The Advertising and Promotional Labeling Branch (APLB) reviewed the proposed instructions for use, package, and container labels on December 21, 2023, and found them acceptable from a promotional and comprehension perspective.

13. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action

The Review Committee reviewed the original submission and related amendments. All review issues have been resolved and therefore the Review Committee recommends licensure of **cobas** Malaria for use on the **cobas** 6800/8800 system.

b) Risk/Benefit Assessment

The **cobas** Malaria is intended for detection of *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* DNA and RNA in whole blood samples from individual human donors. The clinical sensitivity of the **cobas** Malaria was 100% for overall (clinical and contrived combined) neat known *Plasmodium*-positive samples with a two-sided 95% exact CI for sensitivity of 99.1%, 100%, suggesting a low probability of false negative results. The clinical specificity was 100% with a two-sided 95% exact CI for specificity of 99.98% – 100%, suggesting a low probability of false positive results. Therefore, **cobas** Malaria demonstrated high clinical sensitivity and specificity, and as a donor screening test would improve blood safety and public health by reducing the risk of transfusion-transmitted malaria in the blood supply.

c) Recommendation for Post-marketing Activities

No postmarketing studies have been proposed for this application.