Rx Only

cobas® Malaria

For *in vitro* diagnostic use

IVD

cobas® Malaria – 192	P/N: 09352511190
cobas® Malaria Control Kit	P/N: 09352520190
cobas® NHP Negative Control Kit	P/N: 09051554190
cobas® omni MGP Reagent	P/N: 06997546190
cobas® omni Specimen Diluent	P/N: 06997511190
cobas® omni Lysis Reagent	P/N: 06997538190
cobas® omni Wash Reagent	P/N: 06997503190

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Intended 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.

Summary and explanation of the test

Background

Malaria is caused by the infection of red blood cells with intracellular protozoan parasites of the genus *Plasmodium*. Malaria initially presents as a febrile illness and, if left untreated, can rapidly progress to a life-threatening disease with symptoms including severe anemia, respiratory distress (due to metabolic acidosis), cerebral malaria and multi-organ failure.[1,2]

Five *Plasmodium* species cause malaria in humans (*P. falciparum, P. malariae, P. vivax, P. ovale,* and *P. knowlesi*). Of these, 2 species—*P. falciparum* and *P. vivax*—are the major contributors to human morbidity.[2,3]

Parasites are usually transmitted to people through the bite of an infected female *Anopheles* mosquito. Malaria can also be transmitted via transfusion (transfusion-transmitted malaria [TTM]), when blood or a blood component from a malaria-infected donor is transfused to a patient, or from mother to child during pregnancy or delivery.[3,4] TTM may cause severe clinical symptoms in the recipients, especially in those with no previous exposure to malaria or in individuals who are immunocompromised due to other coexisting diseases.[5]

TTM can occur in both endemic and non-endemic areas. In non-endemic areas, TTM is usually the result of blood or a blood product collected from a donor who was infected during travel to malaria-endemic areas or from chronically infected immigrants from endemic areas.[5,6] A sensitive nucleic acid test (NAT) is an important tool to screen blood donors for the presence of *Plasmodium* parasites.

Rationale for NAT testing

Malaria can be transmitted via transfusion.[3] In endemic areas, the current practice is to use antigen testing or microscopy to screen donations,[4] but these methods lack sufficient sensitivity to detect all potentially infectious units.[7] In some non-endemic countries, antibody tests are used to qualify donors who indicate a malaria risk on their donor screening questionnaire, but these tests do not differentiate between current and past infection. Furthermore, current antibody assays show variable detection and poor agreement.[8,9] **cobas**® Malaria, a nucleic acid test that detects the 5 *Plasmodium* species that cause human disease, provides a highly sensitive and specific method to directly detect donations infected with *Plasmodium*, enabling them to be removed from the blood supply. This provides heightened protection from TTM infection for recipients of donated blood components or products and further improves the safety of the blood supply.

Explanation of the test

cobas® Malaria is a qualitative PCR test for the detection of *Plasmodium* (*P. falciparum, P. malariae, P. vivax, P. ovale* and *P. knowlesi*) DNA and RNA that is run on the **cobas**® 6800 System and **cobas**® 8800 System. **cobas**® Malaria detects five species of Malaria: *Plasmodium falciparum* (most prevalent), *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi*. Of these, 2 species—*P. falciparum* and *P. vivax*—are the major contributors to human morbidity.

Principles of the procedure

cobas® Malaria is based on a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection.

The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 Systems software, which assigns test results for all tests as either non-reactive, reactive or invalid. Results can be reviewed directly on the system screen, and printed as a report.

Samples should be tested as individual samples. Whole blood may be collected in the 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 tube containing the lysed whole blood is the primary tube on the analyzer, on which the universal sample preparation steps will be performed by the **cobas**® 6800/8800 Systems.

Armored RNA internal control (IC) molecules are added during universal sample preparation and serves as a full process control from sample preparation through amplification/detection. The IC monitors for interference that could cause false negative results. Potentially affected samples are invalidated.

The test also utilizes two external controls: a positive and a negative control. In addition to the sample lysis and release of nucleic acid which occurs in the primary tube, nucleic acids are also released by addition of proteinase and lysis reagent to the sample and controls. The released nucleic acids bind to the silica surface of the magnetic glass particles, which are added to the sample. Unbound substances and impurities, such as denatured proteins, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acids are eluted from the glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the donor sample is achieved by the use of specific forward and reverse primers which are selected from highly conserved regions of the target nucleic acid. A thermostable DNA polymerase

enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[10-12] Any contaminating amplicons from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR mix, when heated in the first thermal cycling step. Newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® Malaria master mix contains detection probes which are specific for *Plasmodium* and IC nucleic acid. The specific *Plasmodium* and IC detection probes are each labeled with one of two unique fluorescent dyes which act as a reporter. Each probe also has a second dye which acts as a quencher. The reporter dyes is measured at a defined wavelength, thus permitting detection and discrimination of the amplified *Plasmodium* targets and the IC.[13,14] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dyes are concomitantly increased. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified *Plasmodium* targets and the IC are possible.

Reagents and materials

cobas® Malaria reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

Table 1 cobas® Malaria test

cobas® Malaria test

Store at 2-8°C

192 test cassette (P/N 09352511190)

Kit components	Reagent ingredients	Quantity per kit 192 tests
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase, glycerol EUH210: Safety data sheets available on request. EUH208: Contains Subtilisin from Bacillus subtilis. May produce an allergic reaction.	22.3 mL
Internal Control (IC)	Tris buffer, < 0.05% EDTA, < 0.001% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide	21.2 mL
Elution Buffer (EB)	Tris buffer, 0.2% methyl 4-hydroxybenzoate	21.2 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, potassium hydroxide, < 0.1% sodium azide	7.5 mL
Malaria Master Mix Reagent 2 (Malaria MMX-R2)	Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, Tween 20, EDTA, < 0.06% dATP, dGTP, dCTP, < 0.14% dUTP, < 0.01% upstream and downstream *Plasmodium* and internal control primers, < 0.01% fluorescent-labeled *Plasmodium* probes, < 0.01% fluorescent-labeled internal control probe, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide	9.7 mL

Table 2 **cobas**® Malaria Control Kit

cobas® Malaria Control Kit

Store at 2-8°C

(P/N 09352520190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Malaria Positive Control (Malaria (+) C)	< 0.001% Synthetic (armored) *Plasmodium* RNA encapsulated in MS2 bacteriophage coat protein, normal human plasma, negative for *Plasmodium* DNA and RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	10.4 mL (16 x 0.65 mL)	 **WARNING** H317: May cause an allergic skin reaction. H412: Harmful to aquatic life with long lasting effects. P261: Avoid breathing mist or vapours. P273: Avoid release to the environment. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Table 3 cobas® NHP Negative Control Kit

cobas® NHP Negative Control Kit

Store at 2-8°C

(P/N 09051554190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, *Plasmodium* DNA and RNA not detectable by PCR methods. < 0.1% ProClin® 300 preservative**	16 mL (16 x 1 mL)	 **WARNING** H317: May cause an allergic skin reaction. P261: Avoid breathing mist or vapours. P272: Contaminated work clothing should not be allowed out of the workplace. P280: Wear protective gloves. P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention. P362 + P364: Take off contaminated clothing before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 55965-84-9 reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

cobas® omni reagents for sample preparation

Table 4 cobas® omni reagents for sample preparation

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning[a]
cobas® omni MGP Reagent (MGP) Store at 2–8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl 4-hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas® omni Specimen Diluent (SPEC DIL) Store at 2–8°C (P/N 06997511190)	Tris buffer, 0.1% methyl 4-hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas® omni Lysis Reagent (LYS) Store at 2–8°C (P/N 06997538190)	42.56% (w/w) guanidine thiocyanate[b], 5% (w/v) polydocanol[b], 2% (w/v) dithiothreitol[b], dihydro sodium citrate	4 x 875 mL	 **DANGER** H302: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071: Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection. P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
cobas® omni Wash Reagent (WASH) Store at 15–30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

Note: These reagents are not included in the **cobas**® Malaria test kit. See listing of additional materials required (Table 7).

[a] Product safety labeling primarily follows EU GHS guidance.

[b] Hazardous substance

Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® Malaria - 192	2–8°C
cobas® Malaria Control Kit	2–8°C
cobas® NHP Negative Control Kit	2–8°C
cobas® **omni** Lysis Reagent	2–8°C
cobas® **omni** MGP Reagent	2–8°C
cobas® **omni** Specimen Diluent	2–8°C
cobas® **omni** Wash Reagent	15–30°C

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The systems allow reagents to be used only if all of the conditions shown in Table 6 are met. The systems automatically prevent use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 6 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® Malaria – 192	Date not passed	90 days since loading[a]	Max 40 runs	Max 40 hours
cobas® Malaria Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® NHP Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® **omni** Lysis Reagent	Date not passed	30 days since loading[b]	Not applicable	Not applicable
cobas® **omni** MGP Reagent	Date not passed	30 days since loading[b]	Not applicable	Not applicable
cobas® **omni** Specimen Diluent	Date not passed	30 days since loading[b]	Not applicable	Not applicable
cobas® **omni** Wash Reagent	Date not passed	30 days since loading[b]	Not applicable	Not applicable

[a] Single use reagents

[b] Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Additional materials required

Table 7 Material and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
Roche Whole Blood Collection Tube	08827907001
cobas® **omni** Processing Plate	05534917001
cobas® **omni** Amplification Plate	05534941001
cobas® **omni** Pipette Tips	05534925001
cobas® **omni** Liquid Waste Container	07094388001
cobas® **omni** Lysis Reagent	06997538190
cobas® **omni** MGP Reagent	06997546190
cobas® **omni** Specimen Diluent	06997511190
cobas® **omni** Wash Reagent	06997503190
Solid Waste Bag	07435967001
Solid Waste Container or Solid Waste Bag With Insert	07094361001 or 08030073001

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® Malaria analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system. The **cobas**® **Synergy** software shall be installed, if applicable.

Table 8 Instrumentation

Equipment	P/N
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module for **cobas**® 6800/8800 Systems	06301037001
cobas® **Synergy** software electronic license (**cobas**® 6800/8800 Systems)	09311238001
Hamilton MICROLAB® STAR IVD	04640535001

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- All samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[15,16] Only personnel proficient in handling infectious materials and the use of **cobas**® Malaria and **cobas**® 6800/8800 Systems, should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.6% sodium hypochlorite in distilled or deionized water or follow appropriate site procedures.
- **cobas**® Malaria Control Kit and **cobas**® NHP Negative Control Kit contain plasma derived from human blood. Testing of normal human plasma by PCR methods also showed no detectable *Plasmodium* DNA and RNA. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- The additive in the Roche Whole Blood Collection Tube contains guanidine hydrochloride. Do not allow direct contact between guanidine hydrochloride and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas. If additive containing guanidine hydrochloride is spilled, clean with suitable laboratory detergent and water. If the spilled additive contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.6% sodium hypochlorite.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas**® **omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- The additive in the Roche Whole Blood Collection Tube contains guanidine hydrochloride, a potentially hazardous chemical. Avoid contact of this additive with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® Malaria kits, **cobas**® **omni** MGP Reagent, and **cobas**® **omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas**® **omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® Malaria kits and **cobas**® **omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.6% sodium hypochlorite in distilled or deionized water. Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instruments, follow the instructions in the **cobas**® 6800/8800 Systems User Assistance to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

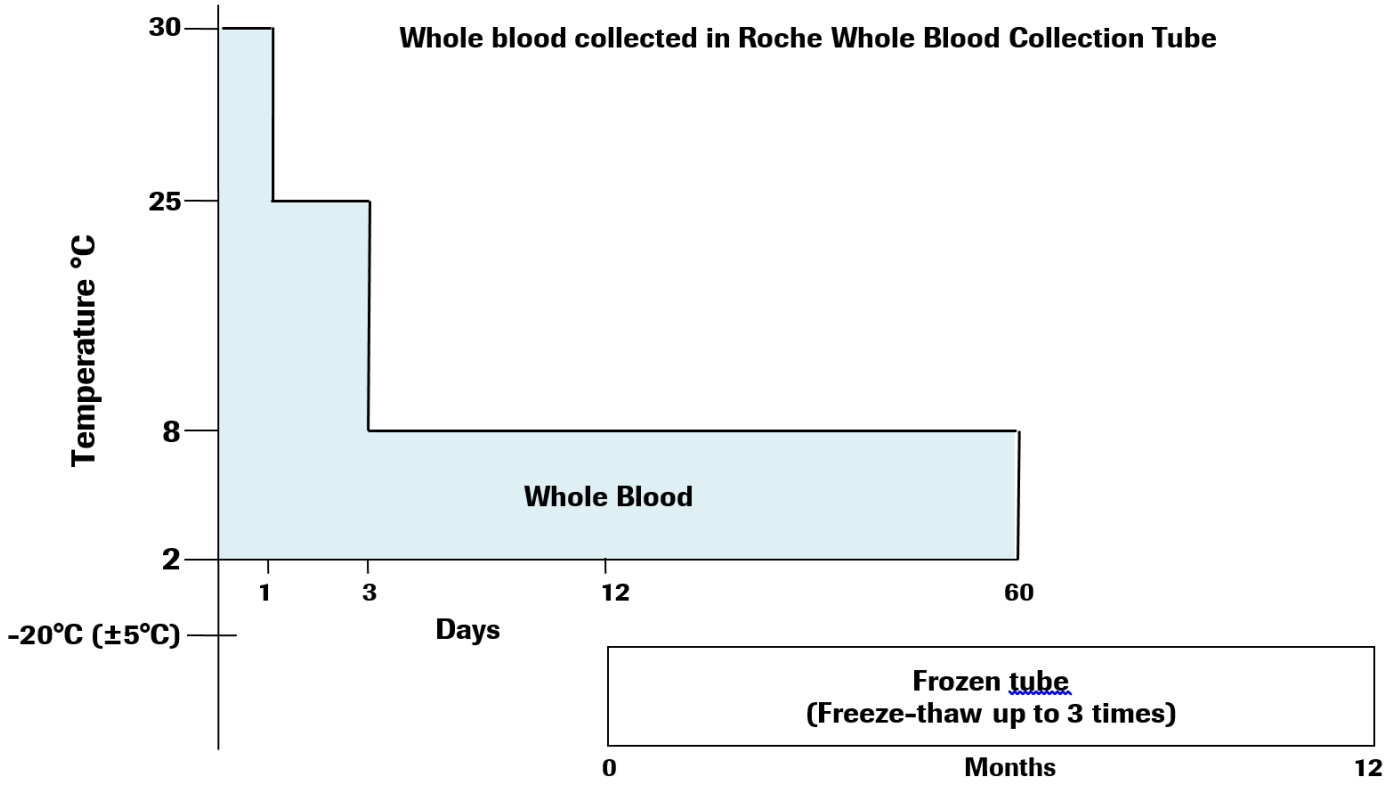
- Store all donor samples at specified temperatures.
- Sample stability is affected by elevated temperatures.
- Centrifuge samples at 1000 RCF (relative centrifugal force) for 2 minutes.

Living donor samples

- Whole blood collected in the Roche Whole Blood Collection Tube may be used with **cobas**® Malaria. Follow the sample collection tube manufacturer instructions for handling and centrifugation.
- Whole blood collected in the Roche Whole Blood Collection Tube may be stored for up to 60 days with the following conditions:
    - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.

Other than noted above, samples are stored at 2-8°C. In addition, the Roche Whole Blood Collection Tube may be stored within the first 12 days after collection for up to 12 months at -20°C (± 5°C) with three freeze/thaw cycles. Refer to Figure 1.

Figure 1 Sample storage conditions for samples collected in the Roche Whole Blood Collection Tube

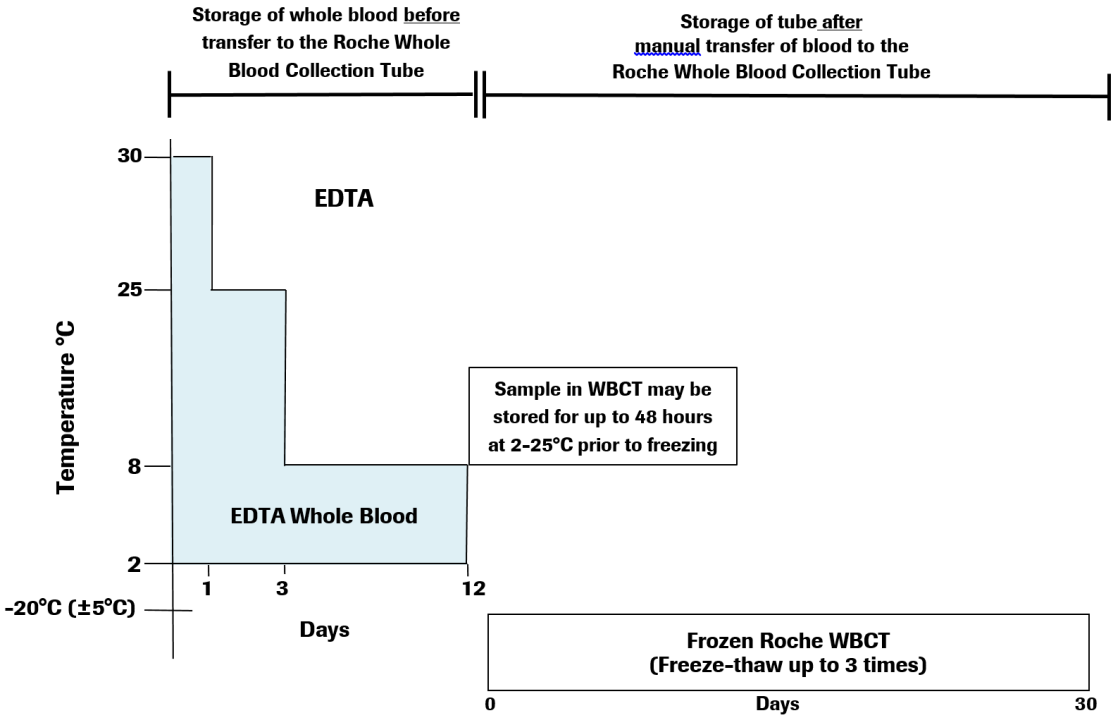


- If the Roche Whole Blood Collection Tube of a donor is not available for testing (e.g., if the tube is damaged or if whole blood was not collected using the Roche Whole Blood Collection Tube), whole blood collected in EDTA anti-coagulant may be used with **cobas**® Malaria.
- Before testing with **cobas**® Malaria, 1.1 mL of EDTA whole blood must be **manually transferred** to the Roche Whole Blood Collection Tube.
- Whole blood collected in EDTA may be stored for up to 12 days prior to dilution in the Roche Whole Blood Collection Tube with the following conditions:
  - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.
  - Other than noted above, samples are stored at 2-8°C. Refer to Figure 2.
- After dilution in the whole blood collection tube, the tube may be stored for up to 48 hours at 2-25°C.

Other than noted above, following dilution in the Roche Whole Blood Collection Tube, specimens are stable at -20°C (+/- 5°C) for 30 days with 3 freeze/thaws when the specimen is frozen within 48 hours of dilution. Refer to Figure 2.

If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

Figure 2 Sample storage conditions for living donor samples collected in EDTA anticoagulant



Instructions for use

Automated sample pipetting (optional)

cobas® **Synergy** software with the Hamilton MICROLAB® STAR IVD can be used as an optional component of the **cobas**® 6800/8800 Systems for automated pipetting. Refer to the **cobas**® **Synergy** software User Assistance for more information.

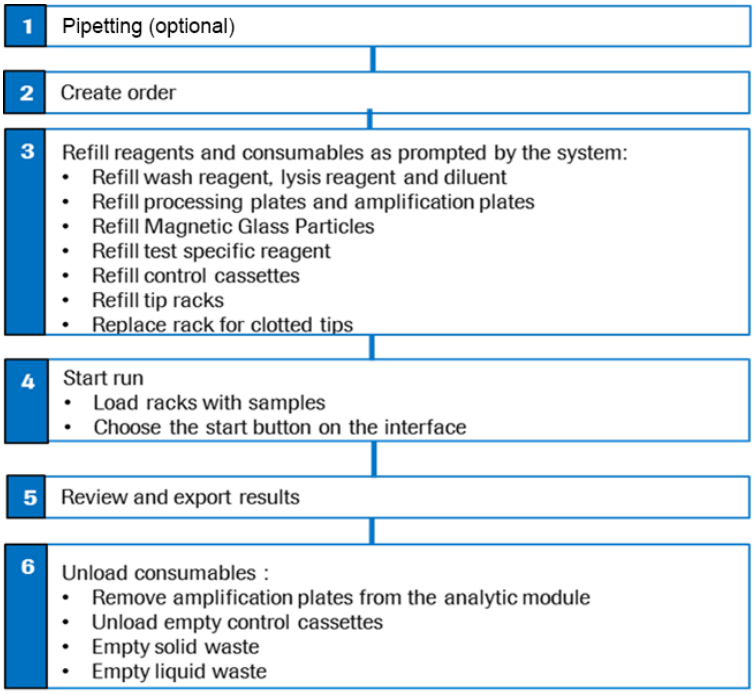
Procedural notes

- Do not use **cobas**® Malaria reagents, **cobas**® Malaria Control Kit, **cobas**® NHP Negative Control Kit or **cobas**® **omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems User Assistance or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional procedures and for proper maintenance of instruments.
- Invalid results may be influenced by a number of contributing factors including, but not limited to, sample characteristics, interfering substances and pre-analytical workflows.
- **cobas**® Malaria test should not be performed using pooled samples.

Running cobas® Malaria

The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance or refer to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pipetting procedures. Figure 3 below summarizes the procedure.

Figure 3 cobas® Malaria procedure



Results

The **cobas**® 6800/8800 Systems automatically detect *Plasmodium* nucleic acid simultaneously for the samples and controls.

Quality control and validity of results

- One negative control [(–) C] and one positive control [Malaria (+) C] are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for either control.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

Control flags

Table 9 Control flags for negative and positive controls

Negative Control	**Flag**	**Result**	**Interpretation**
(-) C	Q02	Invalid	The entire batch is assigned invalid if the result for the (-) C is invalid.
Positive Control	**Flag**	**Result**	**Interpretation**
Malaria (+) C	Q02	Invalid	The entire batch is assigned invalid if the result for the Malaria (+) C is invalid.

If the batch is invalid, repeat testing of the entire batch including samples and controls.

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid donor sample results dependent on flags obtained for the individual samples.
- Sample results are valid only if the respective positive control and the negative control of the corresponding batch are valid.

Two parameters are measured simultaneously for each sample: *Plasmodium* and the internal control. Final sample results for the **cobas**® Malaria test are reported by the software. In addition to the overall results, individual target results will be displayed in the **cobas**® 6800/8800 software and should be interpreted as follows:

Table 10 Target results for individual target result interpretation

Target results	Interpretation
Malaria Non-Reactive	No target signal detected for ***Plasmodium*** and IC signal detected.
Malaria Reactive	Target signal detected for ***Plasmodium*** and IC signal may or may not be detected.
Invalid	Target and internal control signal not detected.

If using the **cobas**® **Synergy** software, review of the final result calculation should be performed through the **cobas**® **Synergy** software.

Repeat testing of individual sample(s)

Sample tubes with a final results of Invalid for the target require repeat testing.

Procedural limitations

- **cobas**® Malaria has been evaluated only for use in combination with the **cobas**® Malaria Control Kit, **cobas**® NHP Negative Control Kit, **cobas**® **omni** MGP Reagent, **cobas**® **omni** Lysis Reagent, **cobas**® **omni** Specimen Diluent, and **cobas**® **omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- **cobas**® Malaria must only be used with whole blood samples collected with or manually added to the Roche Whole Blood Collection tube.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Detection of *Plasmodium* DNA and RNA is dependent on the number of Malaria infected red blood cells present in the sample and may be affected by sample collection, storage and handling, patient factors (i.e., age, presence of symptoms), and stage of infection.
- Though rare, mutations within the highly conserved regions of a *Plasmodium* genome covered by **cobas**® Malaria, may affect primer and/or probe binding resulting in the failure to detect presence of the *Plasmodium* organism.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.
- When blood is collected directly to the Roche WBC tube, the volume may be less than the expected 1.1 ml when blood is collected at high altitude, which could result in a false negative result when parasitemia is close to the LOD of the assay.

Non-clinical performance evaluation performed on the cobas® 6800/8800 Systems

Key performance characteristics

Limit of Detection (LoD)

Analytical sensitivity was determined for *Plasmodium falciparum, P. malariae, P. vivax, P.ovale* and *P. knowlesi.* using different source materials based on availability. Table 12 outlines the materials used for each *Plasmodium* species.

Table 11 Limit of Detection Source Materials by Species

Species	LoD using whole blood sample prior to lysis	LoD using Roche Secondary Standards**	LoD using Armored RNA
Plasmodium falciparum	X	X	X
Plasmodium vivax		X	X
Plasmodium knowlesi		X	X
*Plasmodium ovale**			X
*Plasmodium malariae**			X

*The sensitivity of these species was only determined using armored RNA due to limitations in availability of well characterized live culture/intact RBC samples.
**Secondary standards are infected red blood cells quantitated prior to resuspension in the pre-analytic chaotropic reagent and stored frozen prior to testing.

LoD using whole blood sample prior to lysis

The LoD of **cobas**® Malaria was determined using *Plasmodium falciparum* strain 3D7 infected red blood cells (iRBC) serially diluted in whole blood prior to lysis in the chaotropic reagent.

The stock titer was assigned as percentage parasitemia (*Plasmodium* infected red blood cells per mL, living synchronous ring stage, Giemsa stain).

Three independent dilution series of the infected red blood cell stock were prepared in human whole blood. 1.1 ml aliquots of each concentration were inoculated into a Roche Whole Blood Collection tube and tested by **cobas**® Malaria.

Each dilution series was tested using three lots of **cobas**® Malaria kits with 45 replicates per lot, for a total of 135 replicates per concentration. PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the 50% and 95% LoD, along with the lower and upper limit of 95% confidence intervals (Table 12). The reactivity rates observed in this LoD study with one-sided 95% confidence interval lower bound are summarized in Table 12.

Table 12 Results of PROBIT analysis on LoD data for *Plasmodium* iRBCs in human whole blood

Analyte	50% LoD (iRBC/mL)	95% Confidence Interval for 50% LoD (iRBC/mL)	95% LoD (iRBC/mL)	95% Confidence Interval for 95% LoD (iRBC/mL)
Plasmodium falciparum	0.6	0.5–0.7	2.9	2.4–3.8

Table 13 Reactivity rates summary for *Plasmodium falciparum*

***Plasmodium falciparum* (iRBC/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
10.0	135	135	100.0%	97.8%
5.0	134	135	99.3%	96.5%
2.5	126	135	93.3%	88.7%
1.0	85	135	63.0%	55.6%
0.5	59	135	43.7%	36.5%
0	0	135	0.0%	0.0%

LoD using Roche Secondary Standards

The LoD of **cobas**® Malaria was determined using in-house Secondary Standards for *Plasmodium falciparum* strain 3D7, *Plasmodium vivax* ATCC 30073 strain NICA and *Plasmodium knowlesi* strain A1-H.1. Secondary standards are infected red blood cells quantitated prior to resuspension in the pre-analytic chaotropic reagent and stored frozen prior to testing.

Secondary Standards were used to prepare 3 independent dilution series using a whole blood chaotropic reagent mixture, simulating the final sample. The dilution series were tested across three reagent lots using 65-66 replicates per lot, for a total of 197-198 replicates per concentration. PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the 50% and 95% LoD reported as iRBC/mL of whole blood, along with the lower and upper limit of 95% confidence intervals (Table 14). The reactivity rates observed in this LoD study with one-sided 95% confidence interval lower bound are summarized in Table 15 to Table 17.

Table 14 Results of PROBIT analysis on LoD data collected with lysed *Plasmodium* iRBCs diluted in whole blood/chaotropic reagent mixture

Analyte	**50% LoD (iRBC/mL)**	**95% Confidence Interval for 50% LoD (iRBC/mL)**	**95% LoD (iRBC/mL)**	**95% Confidence Interval for 95% LoD (iRBC/mL)**
Plasmodium falciparum	0.013	0.011 – 0.014	0.058	0.049 – 0.071
Plasmodium vivax	0.003	0.002 – 0.003	0.012	0.010 – 0.015
Plasmodium knowlesi	0.009	0.008 – 0.011	0.044	0.037 – 0.054

Table 15 Reactivity rates summary for *Plasmodium falciparum* Secondary Standard

***Plasmodium falciparum* (iRBC/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
0.130	197	197	100.0%	98.5%
0.052	187	198	94.4%	91.0%
0.026	151	198	76.3%	70.8%
0.013	94	198	47.5%	41.4%
0.007	60	198	30.3%	24.9%
0	0	198	0.0%	0.0%

Table 16 Reactivity rates summary for *Plasmodium vivax* Secondary Standard

Plasmodium vivax **(iRBC/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
0.033	198	198	100.0%	98.5%
0.013	194	198	98.0%	95.4%
0.007	158	198	79.8%	74.5%
0.003	111	198	56.1%	50.0%
0.002	77	198	38.9%	33.1%
0	0	198	0.0%	0.0%

Table 17 Reactivity rates summary for *Plasmodium knowlesi* Secondary Standard

Plasmodium knowlesi **(iRBC/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
0.195	198	198	100.0%	98.5%
0.078	196	198	99.0%	96.9%
0.039	186	198	94.0%	90.4%
0.020	153	198	77.3%	71.8%
0.010	108	198	54.6%	48.5%
0	0	198	0.0%	0.0%

LoD using Armored RNA

The LoD of **cobas**® Malaria for the 5 *Plasmodium* species was determined using armored RNA sequences in **cobas**® **omni** Specimen Diluent. The armored RNA sequences correspond to the target regions of the 18S ribosomal RNA of *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale* and *Plasmodium knowlesi*.

One dilution series in **cobas**® **omni** Specimen Diluent was prepared for each material and tested using three different lots of **cobas**® Malaria kits with 23-24 replicates per lot, for a total of 71-72 replicates per concentration. PROBIT analysis on the data combined across reagent lots was used to estimate the 50% and 95% LoD, along with the lower and upper limit of 95% confidence intervals (Table 18). The reactivity rates observed in this LoD study with one-sided 95% confidence interval lower bound are summarized in Table 19 to Table 23.

Table 18 Results of PROBIT analysis on LoD data collected with *Plasmodium* armored RNA in **cobas**® **omni** Specimen Diluent

Analyte	50% LoD (armored particles/mL)	95% Confidence Interval for 50% LoD (armored particles/mL)	95% LoD (armored particles/mL)	95% Confidence Interval for 95% LoD (armored particles/mL)
Plasmodium falciparum	8.2	6.8 – 9.5	27.9	22.5 – 38.5
Plasmodium malariae	9.0	7.5 – 10.4	32.2	25.8 – 44.7
Plasmodium vivax	8.2	6.7 – 9.7	33.1	26.3 – 46.5
Plasmodium ovale	9.5	7.5 – 11.4	59.0	44.1 – 90.3
Plasmodium knowlesi	6.6	5.2 – 7.8	23.7	18.9 – 33.6

Table 19 Reactivity rates summary for *Plasmodium falciparum* armored RNA in **cobas**® **omni** Specimen Diluent

Plasmodium falciparum **(armored particles/mL)**	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
100	72	72	100.0%	95.9%
50	72	72	100.0%	95.9%
25	66	72	91.7%	84.2%
12	50	72	69.4%	59.3%
6	25	72	34.7%	25.4%
0	0	72	0.0%	0.0%

Table 20 Reactivity rates summary for *Plasmodium malariae* armored RNA in **cobas**® **omni** Specimen Diluent

Plasmodium malariae **(armored particles/mL)**	Number reactive	Number of valid replicates	% Reactive	95% Lower confidence bound (one-sided)
100	72	72	100.0%	95.9%
50	72	72	100.0%	95.9%
25	64	71	90.1%	82.3%
12	42	72	58.3%	48.0%
6	25	72	34.7%	25.4%
0	0	72	0.0%	0.0%

Table 21 Reactivity rates summary for *Plasmodium vivax* armored RNA in **cobas**® **omni** Specimen Diluent

Plasmodium vivax **(armored particles/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
100	72	72	100.0%	95.9%
50	71	72	98.6%	93.6%
25	63	72	87.5%	79.2%
12	52	72	72.2%	62.2%
6	24	72	33.3%	24.2%
0	0	72	0.0%	0.0%

Table 22 Reactivity rates summary for *Plasmodium ovale* armored RNA in **cobas**® **omni** Specimen Diluent

Plasmodium ovale **(armored particles/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
100	72	72	100.0%	95.9%
50	69	72	95.8%	89.6%
25	52	72	72.2%	62.2%
12	41	72	56.9%	46.6%
6	28	72	38.9%	29.2%
0	0	72	0.0%	0.0%

Table 23 Reactivity rates summary for *Plasmodium knowlesi* armored RNA in **cobas**® **omni** Specimen Diluent

Plasmodium knowlesi **(armored particles/mL)**	**Number reactive**	**Number of valid replicates**	**% Reactive**	**95% Lower confidence bound (one-sided)**
100	72	72	100.0%	95.9%
50	72	72	100.0%	95.9%
25	69	72	95.8%	89.6%
12	54	72	75.0%	65.2%
6	34	72	47.2%	37.1%
0	0	71	0.0%	0.0%

Repeatability/Precision

Repeatability/precision was calculated from the mean, SD, and % CV of Cycle threshold (Ct) values for positive panel members with reactive **cobas**® Malaria results overall (within-laboratory precision) by *Plasmodium* species and expected *Plasmodium* concentration. The highest within-laboratory % CV observed was 3.0% for *P. vivax* at ~0.5× LoD. The lowest within-laboratory % CV observed was 1.6% for *P. falciparum* at ~2.5× LoD. The within-laboratory % CV for all other positive panel members from all species were ≤ 2.8%. Within each species, the within-laboratory % CV decreased as the target parasite concentration increased from ~0.5× LoD to ~2.5× LoD, as expected. Results are summarized in Table 24.

Table 24 Within Laboratory Precision (Repeatability) by *Plasmodium* Species

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

SD = Standard Deviation, % CV = percent coefficient of variation, iRBC = infected red blood cells, LoD = limit of detection.

* 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.

Genotype verification

The ability of **cobas**® Malaria to detect 5 species of *Plasmodium* was also shown by testing a total of 10 unique clinical samples each for *Plasmodium falciparum, P. malariae, P. vivax* and *P. ovale*, and the in-house Secondary Standard for *Plasmodium knowlesi strain A1-H.1*. Each clinical sample was tested as 1 replicate neat and 1 replicate after dilution in *Plasmodium* negative human whole blood to approximately 3 x LoD of **cobas**® Malaria. The *Plasmodium knowlesi* standard was tested as 1 replicate each after dilution to approximately 3 x LoD in 10 *Plasmodium* negative human whole blood samples. All samples and cultures were detected neat and at approximately 3 x LoD.

Sensitivity assessment using clinical specimens

The clinical sensitivity of **cobas**® Malaria was evaluated in-house using 100 individual clinical samples (61 *P. falciparum* and 39 *P. vivax*) that were known to be *Plasmodium*-positive based on microscopy testing. All samples were quantified traceable to the *Plasmodium falciparum* or *Plasmodium vivax* Roche Secondary Standards. Each clinical sample was tested singly after dilution in a mixture of *Plasmodium* negative human whole blood and chaotropic reagent to approximately 5 x LoD and 3 x LoD of **cobas**® Malaria (Table 25). All samples were detected as Reactive.

Table 25 Clinical Sensitivity for *Plasmodium falciparum* and *vivax*

Species	Concentration	Number Reactive/ Total Samples	% Sensitivity (95% CI)
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%)

Note: CI = two-sided Clopper-Pearson (exact) binomial confidence interval

Specificity assessment using clinical specimens

The specificity of the **cobas®** Malaria test with clinical specimens was evaluated using 500 individual Malaria 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.

Analytical specificity

Analytical specificity – cross reactivity

The analytical specificity of **cobas**® Malaria was evaluated for cross-reactivity with 16 microorganisms at 10^5 - 10^6 copies, genome copies, cells, CFU or IU/mL, which included 6 viral isolates, 1 parasite, 8 bacterial strains and 1 yeast isolate (Table 26). The microorganisms (up to 5 clinical samples and/or 1 culture each) were added to *Plasmodium*-negative human whole blood and tested with and without *Plasmodium falciparum* secondary standard added to a concentration of approximately 3 x LoD of **cobas**® Malaria. The tested microorganisms do not cross-react or interfere with **cobas**® Malaria.

Table 26 Microorganisms tested for analytical specificity

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	-

Analytical specificity – interfering substances

Endogenous interference substances

Whole blood samples with abnormally high levels of triglycerides (33 g/L), hemoglobin (≥ 200 g/L), unconjugated bilirubin (684 µmol/L), albumin (60 g/L), and human DNA (2 mg/L) were tested with and without *Plasmodium falciparum* secondary standard added to a concentration of approximately 3 x LoD of **cobas**® Malaria. Samples containing these endogenous substances did not cross-react or interfere with the **cobas**® Malaria.

Exogenous interference substances

Plasmodium-negative human whole blood samples containing abnormally high concentrations of drugs (Table 27) were tested with and without P*lasmodium falciparum* secondary standard added to a concentration of 3 x LoD of **cobas**® Malaria. These exogenous substances did not cross-react or interfere with the **cobas**® Malaria.

Table 27 Concentrations of the drugs added into whole blood

Name of drug tested	Concentration
Acetaminophen	1324 µmol/L
Acetylsalicylic Acid	3620 µmol/L
Ascorbic Acid	342 µmol/L
Atenolol	33.8 µmol/L
Atorvastatin	1.34 µmol/L
Atovaquone	1227 µmol/L
Azithromycin	15.3 µmol/L
Fluoxetine	11.2 µmol/L
Ibuprofen	2425 µmol/L
Loratadine	0.78 µmol/L
Naproxen	2170 µmol/L
Paroxetine	3.63 µmol/L
Phenylephrine HCl	491 µmol/L
Sertraline	3.03 µmol/L

Whole System Failure

The whole system failure rate of **cobas**® Malaria was determined by testing 100 replicates of whole blood spiked with *Plasmodium falciparum* secondary standard at a target concentration of approximately 3 x LoD. Before testing with **cobas**® Malaria, each panel member was diluted in the Roche Whole Blood Collection Tube. The results of this study determined that all replicates were reactive, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.6% for the upper bound.

Clinical performance evaluation

Clinical sensitivity

The clinical sensitivity of **cobas**® Malaria was evaluated using 417 individual samples (237 clinical samples (*P. falciparum*, *P. vivax*, and *P. malariae*) and 180 contrived samples (*P. falciparum*, *P. malariae*, *P. vivax*, *P. ovale*, and *P. knowlesi*) that were known to be *Plasmodium*-positive based on NAT testing. The study was conducted at three testing laboratories, with each site testing approximately one third of the samples neat using three different lots of **cobas**® Malaria.

The clinical sensitivity of **cobas**® Malaria with neat samples in this study was 100% (417/417; 95% two-sided Clopper-Pearson (exact) binomial Confidence Interval (CI): 99.1% to 100%) (Table 28).

Table 28 Clinical sensitivity of known *Plasmodium*-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%)

Note: CI = two-sided Clopper-Pearson (exact) binomial confidence interval, n/a = not applicable.

Clinical specificity

The clinical specificity of **cobas**® Malaria was evaluated using blood donations (approximately 1 mL of whole blood collected in a Roche Whole Blood Collection Tube) screened at three external laboratory sites. Four different **cobas**® Malaria reagent lots were used in this study. Donors reactive on **cobas** Malaria would have been further evaluated using alternative NAT and *Plasmodium* antibody and invited to participate in a follow-up study. No donations were reactive, therefore no additional testing or follow-up studies were needed. 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. Donations reactive on **cobas**® Malaria would have been defined as status-positive if they were also reactive on an alternative NAT or a Plasmodium antibody test on the index donation or a donor follow-up sample. All evaluable donations that were not status-positive were defined as status-negative.

Clinical specificity of **cobas**® Malaria was calculated as the percentage (95% two-sided CI) of *Plasmodium* status-negative donors who had **cobas**® Malaria non-reactive results. A total of 20,187 evaluable donations were tested as individual samples and 159 evaluable donations from donors deferred from donating blood due to their responses to questions about malaria risk were tested as individual samples.

Table 29 shows the comparison of **cobas**® Malaria results and donation status for 20,187 evaluable donations from which whole blood samples were tested.

Table 29 Comparison of **cobas**® Malaria results with donation status – individual donation testing

cobas® Malaria Result	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

Note: Only evaluable donations are included in this summary table.

* 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.

The clinical specificity for **cobas**® Malaria for donations tested was 100% (20,187/20,187; 95% CI: 99.982% to 100%) (Table 30).

Table 30 Clinical specificity of **cobas**® Malaria – individual donation testing

Parameter	Total Number of Status-Negative Donations	cobas® Malaria Reactive	cobas® Malaria Non-Reactive	Estimate in Percent (95% Exact CI)
Clinical Specificity	20,187	0	20,187	100.000 (99.982, 100.000)

Note: CI = two-sided Clopper-Pearson (exact) binomial confidence interval.

For donor samples that were tested, 339 (99.4%) valid **cobas**® Malaria batches yielded 20,187 (98.92%) valid results. Overall, 99.50% of donations tested contributed valid results after initial testing and retesting, if performed, in the study. Retesting was not performed for 0.43% of donations tested.

Deferred Donors

Table 31 shows the comparison of **cobas**® Malaria results and donation status for 159 evaluable donations from deferred donors that were tested individually. No deferred donors were confirmed positive for *Plasmodium* infection.

Table 31 Comparison of **cobas**® Malaria results with donation status – deferred donors

cobas® Malaria Result	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

Note: Only evaluable donations are included in this summary table.

* 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.

Reproducibility

The reproducibility of **cobas**® Malaria was established by testing a 16-member panel composed of one negative panel member and fifteen samples positive for one of each of five *Plasmodium* species (*P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi*) at three different concentrations (approximately 0.5 x, 1-2 x, and approximately 3 x the LoD **cobas**® Malaria for each of the five *Plasmodium* species). The LoD using Roche Secondary Standards was used for *P. falciparum*, *P. vivax* and *P. knowlesi* positive panel members. The LoD using Armored RNA was used for *P. malariae* and *P. ovale* positive panel members.

Operators at each of three sites performed five days of testing with each of three lots of **cobas**® Malaria reagents and two valid panel runs (i.e., two batches, each batch composed of one panel and two independent controls) per day were completed to yield up to 270 tests per panel member of *Plasmodium* species at each of the three concentrations.

All valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member. Table 32 presents the mean, SD, and % CV of Ct values for positive panel members with reactive **cobas**® Malaria results overall and for each individual variance component (i.e., site, lot, day, between-batch, and within-batch) by expected *Plasmodium* concentration and *Plasmodium* species. The total SD and total % CV are also presented in the last two columns. Little overall variability was observed for the Ct values across site, lot, day, or batch or within-batch for the ~0.5× LoD, 1-2× LoD, and ~3× LoD panel members of each *Plasmodium* species. 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, as expected. This study demonstrated that **cobas**® Malaria for use on the **cobas**® 6800/8800 Systems shows reproducible performance across the variables assessed (lot, site, day, batch, and within batch) for detecting *Plasmodium*.

Table 32 Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Cycle Threshold by *Plasmodium* Species and Expected *Plasmodium* Concentration (Positive Panel Members)

***Plasmodium* Species**	**Expected *Plasmodium* Concentration**	**n*/N**	**Mean Ct**	**Within-Batch SD**	**Within-Batch %CV**	**Between-Batch SD**	**Between-Batch %CV**	**Day SD**	**Day %CV**	**Site SD**	**Site %CV**	**Lot SD**	**Lot %CV**	**Total SD**	**Total %CV**
P. falciparum	~0.5 x LoD	229/270	37.1	0.73	2.0%	0.00	0.0%	0.25	0.7%	0.00	0.0%	0.10	0.3%	0.78	2.1%
P. falciparum	1-2 x LoD	270/270	36.1	0.72	2.0%	0.00	0.0%	0.07	0.2%	0.00	0.0%	0.15	0.4%	0.73	2.0%
P. falciparum	~3 x LoD	270/270	35.4	0.59	1.7%	0.21	0.6%	0.00	0.0%	0.07	0.2%	0.05	0.1%	0.64	1.8%
P. vivax	~0.5 x LoD	251/270	37.0	0.88	2.4%	0.09	0.3%	0.18	0.5%	0.00	0.0%	0.16	0.4%	0.92	2.5%
P. vivax	1-2 x LoD	269/269	35.8	0.70	1.9%	0.08	0.2%	0.00	0.0%	0.01	0.0%	0.00	0.0%	0.70	2.0%
P. vivax	~3 x LoD	269/269	35.0	0.64	1.8%	0.00	0.0%	0.13	0.4%	0.11	0.3%	0.07	0.2%	0.66	1.9%
P. ovale	~0.5 x LoD	211/270	37.3	0.75	2.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.75	2.0%
P. ovale	1-2 x LoD	269/270	36.4	0.68	1.9%	0.10	0.3%	0.00	0.0%	0.06	0.2%	0.00	0.0%	0.69	1.9%
P. ovale	~3 x LoD	270/270	35.5	0.48	1.4%	0.00	0.0%	0.00	0.0%	0.10	0.3%	0.00	0.0%	0.49	1.4%
P. malariae	~0.5 x LoD	210/270	37.6	0.79	2.1%	0.00	0.0%	0.00	0.0%	0.17	0.5%	0.09	0.2%	0.81	2.2%
P. malariae	1-2 x LoD	263/269	36.9	0.59	1.6%	0.14	0.4%	0.00	0.0%	0.03	0.1%	0.18	0.5%	0.64	1.7%
P. malariae	~3 x LoD	270/270	36.1	0.48	1.3%	0.16	0.4%	0.06	0.2%	0.15	0.4%	0.12	0.3%	0.54	1.5%
P. knowlesi	~0.5 x LoD	220/270	37.3	0.81	2.2%	0.27	0.7%	0.00	0.0%	0.07	0.2%	0.09	0.2%	0.86	2.3%
P. knowlesi	1-2 x LoD	270/270	36.3	0.71	2.0%	0.00	0.0%	0.00	0.0%	0.10	0.3%	0.13	0.3%	0.73	2.0%
P. knowlesi	~3 x LoD	270/270	35.5	0.62	1.8%	0.00	0.0%	0.10	0.3%	0.08	0.2%	0.00	0.0%	0.64	1.8%

Note: SD = standard deviation, %CV = percent coefficient of variation, Ct = cycle threshold, LoD = limit of detection.

* 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.33

Asymptomatic population in an endemic area

The positive percent agreement (PPA) of **cobas**® Malaria results with microscopy was evaluated testing whole blood samples collected from 199 healthy individuals from a malaria-endemic region. All samples were collected in Nigeria in August 2021 [41 (20.6%)] and September 2021 [158 (79.4%)]. Alternate NAT (ALT NAT), microscopy, antigen and antibody results were used to determine the status of each specimen.

Of the 199 evaluable samples from subjects, 4 (2.0%; 4/199) were positive on microscopy for *P. falciparum*, positive by antigen testing, reactive on **cobas**® Malaria, and reactive on ALT NAT. Three of these subjects were antibody positive and one was antibody negative. These 4 subjects were classified as status-positive (current infection). The PPA of **cobas**® Malaria with microscopy was 100% (4/4; 95% CI: 51.0% to 100%).

Seventy-two subjects were reactive on **cobas**® Malaria and confirmed by ALT NAT, but negative on microscopy and antigen tests. These were classified as having current infection. Of these, 67 were antibody positive, 2 were antibody negative, and 3 had equivocal antibody results.

Eight subjects were microscopy and antigen negative with discordant results between **cobas**® Malaria and ALT NAT; their status could not be definitively established. Of these, 1 was **cobas**® Malaria reactive and nonreactive by ALT NAT, and 7 were reactive on ALT NAT but nonreactive on **cobas**® Malaria. All 8 were nonreactive on triplicate re-testing by ALT NAT; 2 were reactive on 1 of 3 additional replicates of **cobas**® Malaria. Possible low level of nucleic acid in these subjects may reflect varying states of acquisition and clearance of infections during the malaria risk season in this hyper-endemic region.

A total of 115 subjects were nonreactive by microscopy, antigen, **cobas**® Malaria, and ALT NAT.

Additional information

Key test features

Sample type	Whole blood in Roche Whole Blood Collection Tube
Amount of sample required	850 µL
Amount of sample processed	500 µL

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 34 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer and distributor

Table 35 Manufacturer and distributor

Roche Molecular Systems, Inc.
US Highway 202 South
Branchburg, NJ 08876 USA
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U.S License No. 1636

Distributed by

Roche Diagnostics
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