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Design History File

|                       |                 |                                |  |
|-----------------------|-----------------|--------------------------------|--|
| DHF Number<br>(Title) | DH-04699.01-326 | DHF<br>Title:<br>(Description) | <b>cobas</b> <sup>®</sup> Babesia Nucleic acid test for use on the <b>cobas</b> <sup>®</sup><br>6800/8800 Systems - Instructions for Use (IFU) – USIVD |
| Version               | 1               |                                |  |

| <b>Version</b> | <b>Description</b> |
|----------------|--------------------|
| 1              | Original           |

# cobas<sup>®</sup> Babesia

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Nucleic acid test  
for use on the cobas<sup>®</sup> 6800/8800 Systems

*For in vitro diagnostic use*



|   |                  |
|---|------------------|
| cobas <sup>®</sup> Babesia – 480            | P/N: 08244049190 |
| cobas <sup>®</sup> Babesia Control Kit      | P/N: 08460981190 |
| cobas <sup>®</sup> NHP Negative Control Kit | P/N: 07002220190 |
| 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® Babesia test for use on the cobas® 6800 and cobas® 8800 Systems is a qualitative *in vitro* nucleic acid screening test for the direct detection of *Babesia* (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) DNA and RNA in whole blood samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen organ and tissue donors when donor samples are obtained while the donor's heart is still beating. Whole blood samples from all donors may be screened as individual samples.

This test is not intended for use as an aid in diagnosis of *Babesia* 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

*Babesia* is a protozoan parasite that infects red blood cells (RBCs) and may cause a disease known as babesiosis. Babesiosis may be treated with antibiotics and anti-parasitics. No vaccine is available.<sup>1</sup>

More than a hundred species of *Babesia* have been identified. The bite of a tick is the usual means through which *Babesia* is transmitted, but *Babesia* may also be transmitted by transfusion or from mother to child during pregnancy or delivery. The vast majority of transfusion-associated cases in the United States (US) are due to *Babesia microti*, and approximately 2% of reported cases are due to *Babesia duncani*.<sup>2</sup> Tick-borne transmission of *B. microti* mainly occurs in 7 states in the Northeast (Connecticut, Massachusetts, New Hampshire, New York, and Rhode Island) and the upper Midwest (Minnesota and Wisconsin). Transmission peaks in the warmer months of the year, but, because there are transfusion and congenital risks of transmission, the infection can occur at any time. *B. duncani* is endemic to the West Coast. Two other species, *B. divergens* and *B. venatorum*, also cause human disease but are not endemic in the US. Babesiosis can be transmitted in areas that are not considered at high risk for transmission of the parasite because blood donors may travel to endemic areas.

The number of cases of babesiosis reported in the US in 2011 was 1,124, of which 10 were transfusion-associated.<sup>3</sup> One hundred and sixty-two cases of transfusion-associated babesiosis were reported from 1979-2009, with the rate apparently increasing over time.<sup>2</sup> Although this statistic likely significantly underestimates the true rate of transfusion-associated babesiosis, it makes *Babesia* one of the most-commonly transmitted transfusion-associated infections in the US.<sup>4</sup> Although a history of babesiosis is a basis for indefinite deferral as a blood donor, donors may be unaware that they carry the parasite, may have asymptomatic parasitemia, and may remain infectious for a year or more. Further, the parasite is viable in blood products. The majority of transfusion-associated cases are associated with erythrocytes (including leukoreduced or irradiated units), with a handful of cases due to whole blood-derived platelet transfusion. Prospective testing of 89,153 blood donations in endemic areas of the US yielded a 0.38% positive rate for *Babesia*.<sup>5</sup>

Most cases of babesiosis are asymptomatic, and symptoms, if they occur may include flu-like symptoms (fever, chills, sweats, headache, myalgia, arthralgia) and hemolytic anemia or thrombocytopenia. Babesiosis is potentially life threatening in patients with asplenia, weakened immune systems (e.g., due to cancer, lymphoma, or Acquired Immunodeficiency Syndrome [AIDS]), comorbidities, such as liver or kidney disease, or who are over the age of 50. In these immunocompromised patients, babesiosis can lead to multi-organ dysfunction, disseminated intravascular coagulation, and death can occur.<sup>1</sup>

## Rationale for NAT testing

*Babesia* is usually tick-borne but is also transmissible by transfusion.<sup>5</sup> US blood donations are not currently required to be screened for the presence of *Babesia*. As of January 2019, the FDA has licensed one *Babesia* test for screening blood donors; no pathogen-reduction technologies for red cell components are available in the US. Clinicians may miss the diagnosis of transfusion-associated babesiosis since the clinical presentation is non-specific, and the nationwide distribution of blood products means that cases can occur outside of areas of high *Babesia* prevalence and outside of the peak summer months of tick-borne disease.

Like other infectious diseases for which blood donations are screened, blood donations must be screened with a sensitive assay to detect *Babesia* so that infected units may be interdicted and discarded. cobas® Babesia provides a sensitive and specific method to detect *Babesia* and thereby provide heightened protection from transfusion-transmitted *Babesia* infection for recipients of donated blood components or products and will further improve the safety of the blood supply.

## Explanation of the test

cobas® Babesia is a qualitative PCR test for the detection of *Babesia* DNA and RNA that is run on the cobas® 6800 System and cobas® 8800 System. cobas® Babesia detects four species of *Babesia*, *Babesia microti* (most prevalent in the US), *Babesia duncani*, *Babesia divergens* (most prevalent in Europe) and *Babesia venatorum*.

## Principles of the procedure

cobas® Babesia is based on 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 software which assigns test results for all tests as 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 collected in EDTA may be transferred manually to the Roche Whole Blood Collection Tube. The whole blood collection tube includes a proprietary additive 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 serve as the sample preparation and amplification/detection process control. 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 deoxythymidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).<sup>6-8</sup> 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. However, newly formed amplicons are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The cobas® Babesia master mix contains detection probes which are specific for *Babesia* and IC nucleic acid. The specific *Babesia* and IC detection probes are each labeled with one of two unique fluorescent dyes which acts as a reporter. Each probe also has a second dye which acts as a quencher. The two reporter dyes are measured at defined wavelengths, thus permitting detection and discrimination of the amplified *Babesia* target and the IC.<sup>9,10</sup> 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 dye is concomitantly increased. Since the two specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified *Babesia* target and the IC are possible.



# Reagents and materials

## cobas® Babesia reagents and controls

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



Table 1 cobas® Babesia

| cobas® Babesia<br>Store at 2-8°C<br>480 test cassette (P/N 08244049190) |  |                               |
|---|--|-------------------------------|
| Kit components  | Reagent ingredients  | Quantity per kit<br>480 tests |
| <b>Proteinase Solution (PASE)</b>                                       | Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% (w/v) proteinase<br><br>EUH210: Safety data sheet available on request.<br>EUH208: Contains Subtilisin. May produce an allergic reaction.   | 38 mL                         |
| <b>Internal Control (IC)</b>  | 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   | 38 mL                         |
| <b>Elution Buffer (EB)</b>  | Tris buffer, 0.2% methyl-4 hydroxybenzoate   | 38 mL                         |
| <b>Master Mix Reagent 1 (MMX-R1)</b>                                    | Manganese acetate, potassium hydroxide, < 0.1% sodium azide  | 14.5 mL                       |
| <b>Babesia Master Mix Reagent 2 (MMX-R2)</b>                            | Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, < 0.1% Tween 20, EDTA, < 0.14% dATP, dGTP, dCTP, dUTPs, < 0.01% upstream and downstream <i>Babesia</i> and internal control primers, < 0.01% Fluorescent-labeled <i>Babesia</i> 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 | 17.5 mL                       |

Table 2 cobas® Babesia Control Kit

## cobas® Babesia Control Kit

Store at 2-8°C  
(P/N 08460981190)

| Kit components                                  | Reagent ingredients  | Quantity per kit                  | Safety symbol and warning*  |
|---|--|-----------------------------------|---|
| <b>Babesia Positive Control (Babesia (+) C)</b> | <p>&lt; 0.001% Synthetic (armored) <i>Babesia</i> RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, <i>Babesia</i> DNA and RNA not detectable by PCR methods.</p> <p>0.1% ProClin® 300 preservative**</p> | <p>10.4 mL<br/>(16 x 0.65 mL)</p> |   <p><b>WARNING</b><br/> H317: May cause an allergic skin reaction.<br/> P261: Avoid breathing dust/fume/gas/mist/vapours/spray.<br/> P272: Contaminated work clothing should not be allowed out of the workplace.<br/> P280: Wear protective gloves.<br/> P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.<br/> P362 + P364: Take off contaminated clothing and wash it before reuse.<br/> P501: Dispose of contents/ container to an approved waste disposal plant.<br/> 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1)</p> |



\* 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 07002220190)


| Kit components                                       | Reagent ingredients  | Quantity per kit     | Safety symbol and warning*   |
|--|--|----------------------|--|
| <b>Normal Human Plasma Negative Control (NHP-NC)</b> | Normal human plasma, <i>Babesia</i> DNA and RNA not detectable by PCR methods.<br><br>< 0.1% ProClin® 300 preservative** | 16 mL<br>(16 x 1 mL) |   <p><b>WARNING</b><br/> H317: May cause an allergic skin reaction.<br/> P261: Avoid breathing dust/fume/gas/mist/vapours/spray.<br/> P272: Contaminated work clothing should not be allowed out of the workplace.<br/> P280: Wear protective gloves.<br/> P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.<br/> P362 + P364: Take off contaminated clothing before reuse.<br/> P501: Dispose of contents/ container to an approved waste disposal plant.</p> 55965-84-9 Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (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**   |
|--|--|------------------|---|
| <b>cobas omni M GP Reagent (M GP)</b><br>Store at 2–8°C<br>(P/N 06997546190)         | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide                          | 480 tests        | Not applicable  |
| <b>cobas omni Specimen Diluent (SPEC DIL)</b><br>Store at 2–8°C<br>(P/N 06997511190) | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide  | 4 x 875 mL       | Not applicable  |
| <b>cobas omni Lysis Reagent (LYS)</b><br>Store at 2–8°C<br>(P/N 06997538190)         | 42.56% (w/w) guanidine thiocyanate***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate | 4 x 875 mL       |  <p><b>DANGER</b></p> <p>H302 + H332: Harmful if swallowed or if inhaled.<br/> H314: Causes severe skin burns and eye damage.<br/> H412: Harmful to aquatic life with long lasting effects.<br/> EUH032: Contact with acids liberates very toxic gas.<br/> P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.<br/> P273: Avoid release to the environment.<br/> P280: Wear protective gloves/protective clothing/eye protection/face protection.<br/> P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.<br/> P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor.<br/> 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.</p> <p>593-84-0 Guanidinium thiocyanate<br/> 9002-92-0 Polidocanol<br/> 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol</p> |
| <b>cobas omni Wash Reagent (WASH)</b><br>Store at 15–30°C<br>(P/N 06997503190)       | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate  | 4.2 L            | Not applicable  |

\* These reagents are not included in the cobas Babesia test kit. See listing of additional materials required (Table 7).

\*\* Product safety labeling primarily follows EU GHS guidance

\*\*\*Hazardous substance

## Reagent storage and handling requirements

Opened 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® Babesia - 480            | 2–8°C               |
| cobas® Babesia 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 system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents 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® Babesia – 480            | Date not passed     | 60 days from first usage    | Max 20 runs                                   | Max 20 hours   |
| cobas® Babesia Control Kit      | Date not passed     | Not applicable <sup>a</sup> | Not applicable                                | Max 10 hours   |
| cobas® NHP Negative Control Kit | Date not passed     | Not applicable <sup>a</sup> | Not applicable                                | Max 10 hours   |
| cobas omni Lysis Reagent        | Date not passed     | 30 days since loading*      | Not applicable                                | Not applicable   |
| cobas omni MGP Reagent          | Date not passed     | 30 days since loading*      | Not applicable                                | Not applicable   |
| cobas omni Specimen Diluent     | Date not passed     | 30 days since loading*      | Not applicable                                | Not applicable   |
| cobas omni Wash Reagent         | Date not passed     | 30 days since loading*      | Not applicable                                | Not applicable   |

<sup>a</sup> Single use reagents

\* 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  |
|---|--|
| <b>Roche Whole Blood Collection Tube</b>  | 08827907001  |
| <b>cobas omni</b> Processing Plate  | 05534917001  |
| <b>cobas omni</b> Amplification Plate   | 05534941001  |
| <b>cobas omni</b> Pipette Tips  | 05534925001  |
| <b>cobas omni</b> Liquid Waste Container  | 07094388001  |
| <b>cobas omni</b> Lysis Reagent   | 06997538190  |
| <b>cobas omni</b> MGP Reagent   | 06997546190  |
| <b>cobas omni</b> Specimen Diluent  | 06997511190  |
| <b>cobas omni</b> Wash Reagent  | 06997503190  |
| Solid Waste Bag and Solid Waste Container<br>or<br>Solid Waste Bag With Insert and Kit Drawer | 07435967001 and 07094361001<br>or<br>08030073001 and 08387281001 |
| Solid Waste Bag With Insert (Set of 20)   | 08030073001  |
| Solid Waste Container   | 07094361001  |

## Instrumentation and software required

The **cobas®** 6800/8800 software and **cobas®** Babesia 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                         |
|--|-----------------------------|
| <b>cobas®</b> 6800 System (Option Moveable)      | 05524245001 and 06379672001 |
| <b>cobas®</b> 6800 System (Fix)                  | 05524245001 and 06379664001 |
| <b>cobas®</b> 8800 System                        | 05412722001                 |
| Sample Supply Module                             | 06301037001                 |
| <b>cobas®</b> Synergy Software Dongle (Optional) | 07788339001                 |

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.<sup>11,12</sup> Only personnel proficient in handling infectious materials and the use of cobas® Babesia and cobas® 800/8800 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 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- cobas® Babesia Control Kit and cobas® HPN Negative Control Kit contain plasma derived from human blood. Testing of normal human plasma by PCR methods also showed no detectable *Babesia* 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 0.5% 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.
- **cobasomni** 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®** Babesia kits, **cobasomni** MGP Reagent, and **cobasomni** 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 **cobasomni** 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®** Babesia kits and **cobasomni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls. Change gloves if contaminated by sample, control, or reagents.
- 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 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas®** 800/8800 instruments, follow the instructions in the **cobas®** 800/8800 Systems User Assistance and/or User Guide 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.

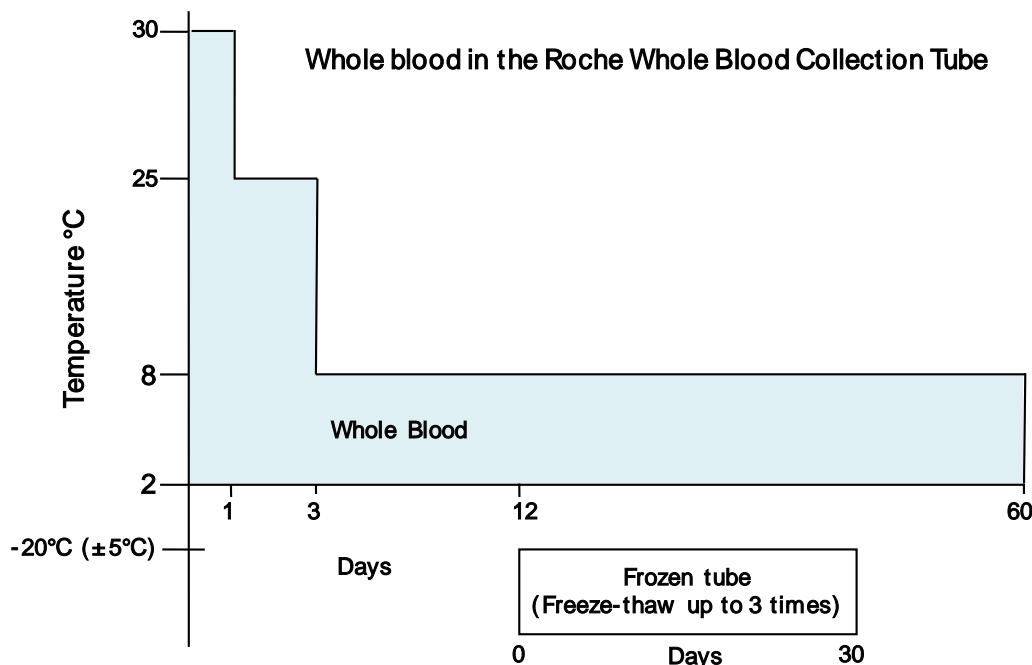
Centrifuge samples at 1000 rcf (relative centrifugal force) for 2 minutes.

### Living donor samples

- Whole blood collected in the Roche Whole Blood Collection Tube may be used with cobas® Babesia. 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.

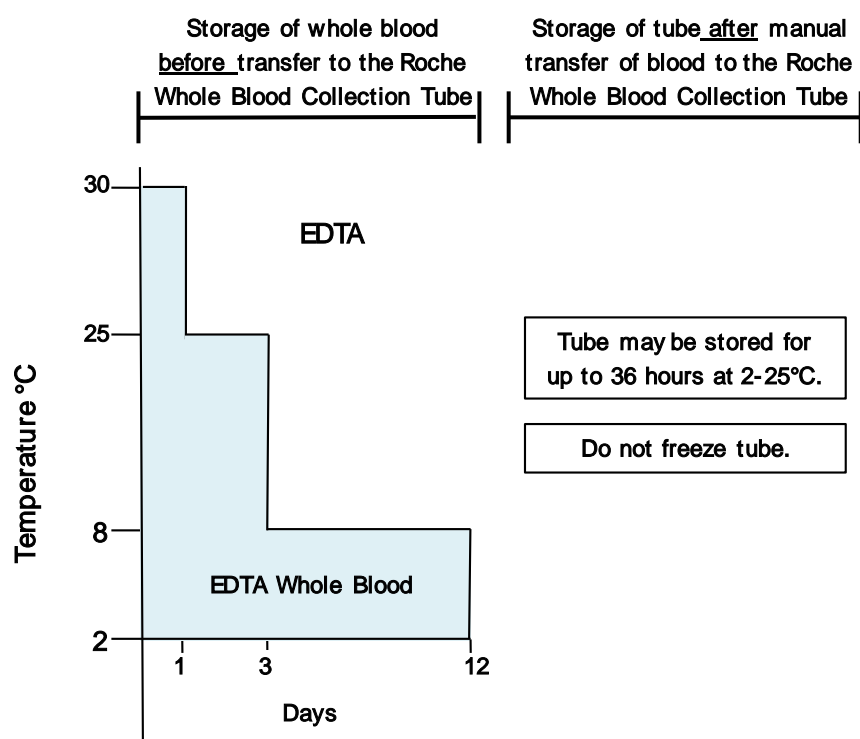
Other than noted above, samples are stored at 2-8°C. In addition the Roche Whole Blood Collection Tube may be stored after 12 days for up to 30 days at -20°C (± 5°C) with three freeze/thaw cycles. Refer to Figure 1.

**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 may be used with cobas® Babesia.
- Before testing with cobas® Babesia 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 36 hours at 2-25°C.

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



- 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.

# Instructions for use

## Procedural notes

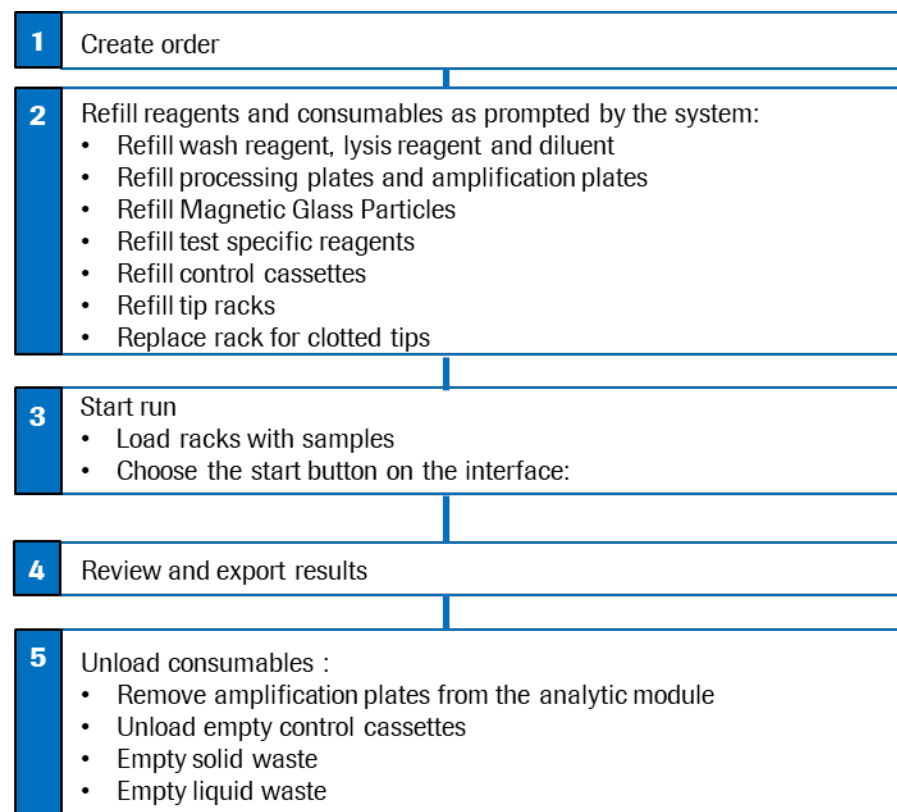
- Do not use **cobas® Babesia** reagents, **cobas® Babesia Control Kit**, **cobas® NHP Negative Control Kit** or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas® 800/8800 Systems User Assistance** and/or **User Guide** for proper maintenance of instruments.
- Refer to the **cobas® Synergy** software **User Assistance** and/or **User Guide** as applicable for further details on optional procedures.

## Running cobas® Babesia

The test procedure is described in detail in the **cobas® 800/8800 Systems User Assistance** and/or **User Guide** or to the **cobas® Synergy** software **User Assistance** and/or **User Guide** as applicable for details on optional procedures.

Figure 3 below summarizes the procedure.

**Figure 3** cobas® Babesia procedure



## Results

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

### Quality control and validity of results

- One negative control [(-) C] and one positive control [Babesia (+) C] are processed with each batch.
- In the cobas® 8800/8800 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 both controls.

Invalidation of results is performed automatically by the cobas® 8800/8800 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   |
| Babesia (+) C    | Q02  | Invalid | The entire batch is assigned invalid if the result for the Babesia (+) 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® 800/8800 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: *Babesia* and the internal control. Final sample results for cobas® Babesia are reported by the software. In addition to the overall results, individual target result will be displayed in the cobas® 800/8800 software and should be interpreted as follows:

**Table 10** Target results for individual target result interpretation

| Target results              | Interpretation   |
|-----------------------------|--|
| <i>Babesia</i> Non-Reactive | No target signal detected for <i>Babesia</i> and IC signal detected.                   |
| <i>Babesia</i> Reactive     | Target signal detected for <i>Babesia</i> and IC signal may be or may not be detected. |
| Invalid                     | Target and internal control signal not detected.                                       |

## Procedural limitations

- cobas® Babesia has been evaluated only for use in combination with the cobas® Babesia Control Kit, cobas® NHP Negative Control Kit, cobasomni MGP Reagent, cobasomni Lysis Reagent, cobasomni Specimen Diluent, and cobasomni Wash Reagent for use on the cobas® 800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Detection of *Babesia* DNA and RNA is dependent on the number of *Babesia* 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/or stage of infection.
- Mutations within the highly conserved regions of a *Babesia* genome covered by cobas® Babesia, may affect primers and/or probe binding resulting in the failure to detect presence of the *Babesia* 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.
- Performance has not been established for cadaveric blood specimens.

# Non-clinical performance evaluation

## Key performance characteristics

### Limit of Detection (LoD)

The limit of detection (LoD) of cobas® Babesia was determined using the following *Babesia* infected red blood cells (iRBC) diluted in human whole blood.

- The *B. microti* infected RBC were obtained from hamster infected with *B. microti* (ATCC, *Babesia microti* Gray, Strain 30221).
- The *B. duncani* infected RBC were obtained from hamster infected with *B. duncani* (ATCC, Strain PRA 302)
- The *B. divergens* infected RBC were obtained from fresh infected sheep blood with *B. divergens* (Oniris, Strain B128)
- The *B. venatorum* infected RBC were obtained from fresh infected sheep blood with *B. venatorum* (Oniris, Strain C201)

The stock titer was provided by the vendor and it was assigned as percentage parasitemia (*Babesia* infected red blood cells per mL, Giemsa stain).

For each of the infected red blood cells stocks, 3 independent dilution series were prepared in human whole blood. Before testing with cobas® Babesia each panel member was diluted in the Roche Whole Blood Collection Tube containing a pre-analytic chaotropic reagent, a guanidine based additive used to lyse the cells within the whole blood, releasing and preserving nucleic acids.

Each dilution series was tested using three different lots of cobas® Babesia kits with approximately 42 replicates per lot, for a total of approximately 126 replicates per concentration. For each *Babesia* species, PROBIT analysis on the data combined across dilution series and reagent lots was used to estimate the LoD, along with the lower and upper limit of 95% confidence interval (Table 11). The reactivity rates observed in the LoD studies for *Babesia* are summarized in Table 12 to Table 15.

**Table 11** Results of PROBIT analysis on LoD data collected with *Babesia* infected red blood cells in human whole blood

| Analyte                  | Measuring units | LoD  | Lower 95% confidence limit | Upper 95% confidence limit |
|--------------------------|-----------------|------|----------------------------|----------------------------|
| <i>Babesia microti</i>   | iRBC/mL         | 6.1  | 5.0                        | 7.9                        |
| <i>Babesia duncani</i>   | iRBC/mL         | 50.2 | 44.2                       | 58.8                       |
| <i>Babesia divergens</i> | iRBC/mL         | 26.1 | 22.3                       | 31.8                       |
| <i>Babesia venatorum</i> | iRBC/mL         | 40.0 | 34.1                       | 48.7                       |

**Table 12** Reactivity rates summary for *Babesia microti*

| <i>Babesia</i> concentration (iRBC/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|--|-----------------|----------------------------|------------|--|
| 11.8                                   | 126             | 126                        | 100.0%     | 97.7%                                  |
| 5.9                                    | 119             | 126                        | 94.4%      | 89.8%                                  |
| 3.0                                    | 103             | 126                        | 81.7%      | 75.1%                                  |
| 1.5                                    | 68              | 126                        | 54.0%      | 46.3%                                  |
| 0.6                                    | 33              | 125                        | 26.4%      | 20.0%                                  |

**Table 13** Reactivity rates summary for *Babesia duncani*

| <i>Babesia</i> concentration (iRBC/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|--|-----------------|----------------------------|------------|--|
| 80.0                                   | 126             | 126                        | 100.0%     | 97.7%                                  |
| 40.0                                   | 115             | 126                        | 91.3%      | 86.0%                                  |
| 20.0                                   | 47              | 126                        | 37.3%      | 30.1%                                  |
| 10.0                                   | 8               | 126                        | 6.3%       | 3.2%                                   |
| 5.0                                    | 2               | 126                        | 1.6%       | 0.3%                                   |

**Table 14** Reactivity rates summary for *Babesia divergens*

| <i>Babesia</i> concentration (iRBC/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|--|-----------------|----------------------------|------------|--|
| 40.0                                   | 126             | 126                        | 100.0%     | 97.7%                                  |
| 20.0                                   | 119             | 126                        | 94.4%      | 89.8%                                  |
| 10.0                                   | 63              | 125                        | 50.4%      | 42.7%                                  |
| 5.0                                    | 26              | 126                        | 20.6%      | 14.9%                                  |
| 2.5                                    | 12              | 126                        | 9.5%       | 5.6%                                   |

**Table 15** Reactivity rates summary for *Babesia venatorum*

| <i>Babesia</i> concentration (iRBC/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|--|-----------------|----------------------------|------------|--|
| 40.0                                   | 124             | 126                        | 98.4%      | 95.1%                                  |
| 20.0                                   | 90              | 126                        | 71.4%      | 64.1%                                  |
| 10.0                                   | 38              | 126                        | 30.2%      | 23.4%                                  |
| 5.0                                    | 9               | 126                        | 7.1%       | 3.8%                                   |
| 2.5                                    | 4               | 126                        | 3.2%       | 1.1%                                   |

## Genotype verification

The performance of cobas® Babesia to detect 4 species of *Babesia* was determined by testing a total of 10 unique clinical samples for *Babesia microti* and 3 *Babesia* cultured isolates. All clinical samples were quantified traceable to the *Babesia microti* Roche Secondary Standard. All clinical samples were tested neat and after dilution with *Babesia* negative human whole blood to 4 x LoD of cobas® Babesia. All 3 *Babesia* cultures were tested after dilution with *Babesia* negative human whole blood to 4 x LoD of cobas® Babesia. All clinical samples and cultures were detected neat and/or at 4 x LoD.

## Analytical specificity

The analytical specificity of cobas® Babesia was evaluated for cross-reactivity with 15 microorganisms at  $10^5$  -  $10^6$  copies, CFU or IU/mL, which included 5 viral isolates, 1 parasite, 8 bacterial strains and 1 yeast isolate (Table 16). The microorganisms were added to *Babesia* negative human whole blood and tested with and without *Babesia* added to a concentration of approximately 3x LoD of cobas® Babesia. The tested microorganisms do not cross-react or interfere with cobas® Babesia.

**Table 16** Microorganisms tested for analytical specificity

| Bacteria                          | Viruses                      | Parasites                    | Yeast                   |
|-----------------------------------|------------------------------|------------------------------|-------------------------|
| <i>Anaplasma phagocytophilum</i>  | Hepatitis B Virus            | <i>Plasmodium falciparum</i> | <i>Candida albicans</i> |
| <i>Propionibacterium acnes</i>    | Hepatitis C Virus            | -                            | -                       |
| <i>Staphylococcus aureus</i>      | Human Immunodeficiency Virus | -                            | -                       |
| <i>Staphylococcus epidermidis</i> | Parvovirus B19               | -                            | -                       |
| <i>Borrelia burgdorferi</i>       | West Nile Virus              | -                            | -                       |
| <i>Borrelia hermsii</i>           | -                            | -                            | -                       |
| <i>Borrelia parkeri</i>           | -                            | -                            | -                       |
| <i>Borrelia recurrentis</i>       | -                            | -                            | -                       |



## Analytical specificity – interfering substances

### Endogenous interference substances

Whole blood samples with abnormally high levels of triglycerides (33 g/L), hemoglobin ( $\geq 20$  g/dL), unconjugated bilirubin (0.2 g/L), albumin (60 g/L), and human DNA (0.002 g/L) were tested with and without *Babesia* added to a concentration of 3 x LoD of cobas® Babesia. Samples containing these endogenous substances did not interfere with the sensitivity or specificity of cobas® Babesia.

### Exogenous interference substances

*Babesia*-negative human whole blood samples containing abnormally high concentrations of drugs (Table 17) were tested with and without *Babesia* added to a concentration of 3 x LoD of cobas® Babesia. These exogenous substances did not interfere with the sensitivity or specificity of cobas® Babesia.

**Table 17** 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    |
| Atorvastatin         | 600 µg Eq/L   |
| Atovaquone           | 1227 µmol/L   |
| Azithromycin         | 15.3 µmol/L   |
| Fluoxetine           | 11.2 µmol/L   |
| Ibuprofen            | 2425 µmol/L   |
| Loratadine           | 0.78 µmol/L   |
| Nadolol              | 3.88 µmol/L   |
| Naproxen             | 2170 µmol/L   |
| Paroxetine           | 3.04 µmol/L   |
| Phenylephrine HCL    | 491 µmol/L    |
| Sertraline           | 1.96 µmol/L   |

## Cross contamination

The cross-contamination rate for cobas® Babesia was determined by testing 237 replicates of *Babesia*-negative human whole blood and 230 replicates of a high titer *Babesia* sample at 1.00E+07 p/mL. The study was performed using the cobas® 800 System. In total, 5 runs were performed with positive and negative samples in a checkerboard configuration.

All 237 replicates of the negative sample were non-reactive, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.54% for the upper bound [0%: 1.54%].

# Clinical performance evaluation

## Clinical sensitivity

The clinical sensitivity of cobas® Babesia was evaluated using 203 individual samples (131 clinical samples (*B. microti*) and 72 contrived samples (*B. microti*, *B. duncani*, *B. venatorum*, and *B. divergens*) that were known to be *Babesia*-positive based on NAT testing. These samples were characterized with a validated in-house NAT for *Babesia*, which used different primers and probes than those used in cobas® Babesia. The study was conducted at three testing laboratories, with each site testing all 203 samples, both neat and diluted 1:6 (to simulate pools of 6), using three different lots of cobas® Babesia.

The clinical sensitivity of cobas® Babesia with neat samples in this study was 100% (95% two-sided Confidence Interval (CI): 98.2% to 100%) and with samples diluted 1:6 was 100% (95% CI: 98.2% to 100%) (Table 18).

**Table 18** Clinical sensitivity of known *Babesia*-positive samples

|      | Number of Samples Tested | Number of Samples Reactive | Number of Samples Non-Reactive | Sensitivity (%) | Sensitivity (95% CI*) |             |
|------|--------------------------|----------------------------|--------------------------------|-----------------|-----------------------|-------------|
|      |                          |                            |                                |                 | Lower Limit           | Upper Limit |
| Neat | 203                      | 203                        | 0                              | 100.0%          | 98.2%                 | 100.0%      |
| 1:6  | 203                      | 203                        | 0                              | 100.0%          | 98.2%                 | 100.0%      |

\*Clopper-Pearson Exact method

## Clinical specificity

The clinical specificity of cobas® Babesia was evaluated testing blood donations collected at five external laboratory sites. Samples were collected in US states classified as high-endemic, low-endemic, or non-endemic for *Babesia*. Six different cobas® Babesia reagent lots were used in this study. Clinical specificity of cobas® Babesia was calculated as the percentage (95% two-sided CI) of *Babesia* donor status-negative donors who had cobas® Babesia non-reactive results. A total of 168,981 evaluable donations were tested as individual samples. The majority of evaluable donations (143,939) were collected in high-endemic US states.

## Individual testing results

Table 19 shows the calculation of the clinical specificity of cobas® Babesia for overall 168,972 evaluable status-negative donations from individual testing, as well as for high-, low-, and non-endemic US states. The clinical specificity of cobas® Babesia overall—across all endemicity categories for donations tested individually—was 99.999% (168,970/168,972; 95% CI: 99.996% to 100%) (Table 19). Specificity results were similar—99.999% to 100%—across the 3 endemicity categories (non-, low- and high-endemic). An invalid rate of 0.49% due to internal control failures, instrument failures, protocol deviations, or other incidents was observed for the individual samples.

**Table 19** Clinical specificity of cobas® Babesia – Overall and per *Babesia* endemicity level

|                     | Parameter                   | Total Number of Status-Negative Donations* | cobas® Babesia Result |              | Estimate in Percent (95% Exact CI) |
|---------------------|-----------------------------|--|-----------------------|--------------|------------------------------------|
|                     |                             |  | Reactive              | Non-Reactive |                                    |
| <b>Overall</b>      | <b>Clinical Specificity</b> | 168,972                                    | 2                     | 168,970      | 99.999 (99.996, 100.000)           |
| <b>Non Endemic</b>  | <b>Clinical Specificity</b> | 10,824                                     | 0                     | 10,824       | 100.000 (99.966, 100.000)          |
| <b>Low Endemic</b>  | <b>Clinical Specificity</b> | 14,217                                     | 0                     | 14,217       | 100.000 (99.974, 100.000)          |
| <b>High Endemic</b> | <b>Clinical Specificity</b> | 143,931                                    | 2                     | 143,929      | 99.999 (99.995, 100.000)           |

Note: Only evaluable donations are included in this summary table. CI = two-sided exact binomial confidence interval.

Table 20 shows the comparison of cobas® Babesia results and donation status for 168,981 evaluable donations, overall and for the three different endemicity levels. Nine (of 11) cobas® Babesia-reactive donations were confirmed positive for *Babesia*, including 8 donations collected in US states determined to be high endemic for *Babesia*.

**Table 20** Comparison of cobas® Babesia results with donation status by endemicity – individual donation testing

|                     | cobas® Babesia Result | Donation Status* |                  | Total N |
|---------------------|-----------------------|------------------|------------------|---------|
|                     |                       | Positive n (%)   | Negative n (%)   |         |
| <b>Overall</b>      | Reactive              | 9 (100.000)      | 2 (0.001)        | 11      |
|                     | Non-Reactive          | 0 (0.000)        | 168,970 (99.999) | 168,970 |
|                     | Total                 | 9                | 168,972          | 168,981 |
| <b>Non Endemic</b>  | Reactive              | 1 (100.000)      | 0 (0.000)        | 1       |
|                     | Non-Reactive          | 0 (0.000)        | 10,824 (100.000) | 10,824  |
|                     | Total                 | 1                | 10,824           | 10,825  |
| <b>Low Endemic</b>  | Reactive              | 0 (0.000)        | 0 (0.000)        | 0       |
|                     | Non-Reactive          | 0 (0.000)        | 14,217 (100.000) | 14,217  |
|                     | Total                 | 0                | 14,217           | 14,217  |
| <b>High Endemic</b> | Reactive              | 8 (100.000)      | 2 (0.001)        | 10      |
|                     | Non-Reactive          | 0 (0.000)        | 143,929 (99.999) | 143,929 |
|                     | Total                 | 8                | 143,931          | 143,939 |

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® Babesia was established by testing a 13-member panel composed of one negative panel member and twelve samples positive for one of each of four *Babesia* species (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) at three different concentrations (approximately 0.5x, 1-2x, and approximately 3x the LoD cobas® Babesia for each of the four species).

Operators at each of three sites performed five days of testing with each of three lots of cobas® Babesia 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 *Babesia* 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 21 (*B. microti*), Table 22 (*B. duncani*), Table 23 (*B. divergens*), and Table 24 (*B. venatorum*)]. This study demonstrated that cobas® Babesia for use on the cobas® 800/8800 System shows reproducible performance across the variables assessed (lot, site, day, batch, and within batch) for detecting *Babesia*.

**Table 21** Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia microti*

| <i>Babesia microti</i><br>Concentration | Site |                          | Lot |                          | Day |                          | Batch |                          |
|---|------|--------------------------|-----|--------------------------|-----|--------------------------|-------|--------------------------|
|   | ID   | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID    | %<br>Reactive<br>Results |
| ~ 0.5 x LoD                             | 1    | 93.3% (84/90)            | 1   | 87.8% (79/90)            | 1   | 96.3% (52/54)            | 1     | 94.8% (128/135)          |
|   | 2    | 96.7% (87/90)            | 2   | 100% (90/90)             | 2   | 94.4% (51/54)            | 2     | 96.3% (130/135)          |
|   | 3    | 96.7% (87/90)            | 3   | 98.9% (89/90)            | 3   | 90.7% (49/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 96.3% (52/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |
| 1-2 x LoD                               | 1    | 100.0% (90/90)           | 1   | 100.0% (89/89)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (134/134)         |
|   | 3    | 100.0% (89/89)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (53/53)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |
| ~ 3 x LoD                               | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |

Note: LoD = Limit of detection.

**Table 22** Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia duncani*

| <i>Babesia duncani</i><br>Concentration | Site |                          | Lot |                          | Day |                          | Batch |                          |
|---|------|--------------------------|-----|--------------------------|-----|--------------------------|-------|--------------------------|
|   | ID   | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID    | %<br>Reactive<br>Results |
| ~ 0.5 x LoD                             | 1    | 46.7% (42/90)            | 1   | 62.2% (56/90)            | 1   | 66.7% (36/54)            | 1     | 65.2% (88/135)           |
|   | 2    | 68.9% (62/90)            | 2   | 54.4% (49/90)            | 2   | 63.0% (34/54)            | 2     | 61.5% (83/135)           |
|   | 3    | 74.4% (67/90)            | 3   | 73.3% (66/90)            | 3   | 57.4% (31/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 64.8% (35/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 64.8% (35/54)            | -     | -                        |
| 1-2 x LoD                               | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (53/53)           | 1     | 100.0% (134/134)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (89/89)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (89/89)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |
| ~ 3 x LoD                               | 1    | 100.0% (90/90)           | 1   | 100.0% (89/89)           | 1   | 100.0% (54/54)           | 1     | 100.0% (134/134)         |
|   | 2    | 100.0% (89/89)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (134/134)         |
|   | 3    | 100.0% (89/89)           | 3   | 100.0% (89/89)           | 3   | 100.0% (53/53)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (53/53)           | -     | -                        |

Note: LoD = Limit of detection.

**Table 23** Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia divergens*

| <i>Babesia divergens</i><br>Concentration | Site |                          | Lot |                          | Day |                          | Batch |                          |
|---|------|--------------------------|-----|--------------------------|-----|--------------------------|-------|--------------------------|
|   | ID   | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID    | %<br>Reactive<br>Results |
| ~ 0.5 x LoD                               | 1    | 35.6% (32/90)            | 1   | 60.0% (54/90)            | 1   | 55.6% (30/54)            | 1     | 52.6% (71/135)           |
|   | 2    | 54.4% (49/90)            | 2   | 28.9% (26/90)            | 2   | 57.4% (31/54)            | 2     | 52.6% (71/135)           |
|   | 3    | 67.8% (61/90)            | 3   | 68.9% (62/90)            | 3   | 46.3% (25/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 51.9% (28/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 51.9% (28/54)            | -     | -                        |
| 1-2 x LoD                                 | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |
| ~ 3 x LoD                                 | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |

Note: LoD = Limit of detection.

**Table 24** Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia venatorum*

| <i>Babesia venatorum</i><br>Concentration | Site |                          | Lot |                          | Day |                          | Batch |                          |
|---|------|--------------------------|-----|--------------------------|-----|--------------------------|-------|--------------------------|
|   | ID   | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID  | %<br>Reactive<br>Results | ID    | %<br>Reactive<br>Results |
| ~ 0.5 x LoD                               | 1    | 95.6% (86/90)            | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 95.6% (86/90)            | 2   | 98.1% (53/54)            | 2     | 97.0% (131/135)          |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 96.3% (52/54)            | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 98.1% (53/54)            | -     | -                        |
| 1-2 x LoD                                 | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |
| ~ 3 x LoD                                 | 1    | 100.0% (90/90)           | 1   | 100.0% (90/90)           | 1   | 100.0% (54/54)           | 1     | 100.0% (135/135)         |
|   | 2    | 100.0% (90/90)           | 2   | 100.0% (90/90)           | 2   | 100.0% (54/54)           | 2     | 100.0% (135/135)         |
|   | 3    | 100.0% (90/90)           | 3   | 100.0% (90/90)           | 3   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 4   | 100.0% (54/54)           | -     | -                        |
|   | -    | -                        | -   | -                        | 5   | 100.0% (54/54)           | -     | -                        |

Note: LoD = Limit of detection.

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## Additional information
























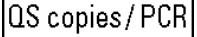


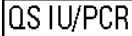





### Key test features

|                                   |  |
|-----------------------------------|--|
| <b>Sample type</b>                | Whole blood in Roche Whole Blood Collection Tube   |
| <b>Amount of sample required</b>  | 850 µL   |
| <b>Amount of sample processed</b> | 500 µL   |
| <b>Test duration</b>              | Results are available within less than 3.5 hours after loading the sample on the system. |



## Symbols

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

|   |   |   |                                       |   |   |
|---|---|---|---------------------------------------|---|---|
|    | Ancillary Software  |    | Lower Limit of Assigned Range         |    | Negative Control  |
|     | Authorized representative in the European community                                   |    | Upper Limit of Assigned Range         |    | Positive Control  |
|    | Barcode Data Sheet  |    | Store in the dark                     |    | Control   |
|    | Batch code  |    | Contains sufficient for $< n >$ tests |    | Assigned Range (copies/mL)  |
|    | Biological risks  |    | Temperature limit                     |    | Assigned Range (IU/mL)  |
|    | Catalogue number  |    | Test Definition File                  |    | Standard Procedure  |
|  | Consult instructions for use  |  | Manufacturer                          |  | Ultrasensitive Procedure  |
|  | Contents of kit   |  | Use-by date                           |  | QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.                             |
|  | Distributed by  |  | Global Trade Item Number              |  | QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.               |
|  | For IVD performance evaluation only   |  | Serial number                         |   | This product fulfills the requirements of the European Directive 98/79 EC for <i>in vitro</i> diagnostic medical devices. |
| Rx Only   | US Only: Federal law restricts this device to sale by or on the order of a physician. |   | Date of manufacture                   |   |   |
|  | <i>In Vitro</i> diagnostic medical device   |  | Do not reuse                          |   |   |

US Customer Technical Support 1-800-526-1247

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