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Rx Only

cobas® Babesia

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

For in vitro diagnostic use

IVD

cobas® Babesia – 480	P/N: 08244049190
cobas® Babesia Control Kit	P/N: 08460981190
cobas® 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 or in pools comprised of aliquots of not more than six 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.[1]

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 *Babesi*a 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*.[2] 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.[3] One hundred and sixty-two cases of transfusion-associated babesiosis were reported from 1979-2009, with the rate apparently increasing over time.[2] Although this statistic likely significantly underestimates the true rate of transfusion-associated babesiosis, it makes *Babesia* one of the most-commonly transmitted transfusion-associated infection in the US.[4] 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*.[5]

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.[1]

Rationale for NAT testing

Babesia is usually tick-borne but is also transmissible by transfusion.[5] 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 or, optionally, can be tested in pools comprised of aliquots of not more than six individual samples. The **cobas**® **Synergy** software with the Hamilton MICROLAB® STAR IVD (**cobas**® **Synergy** Core), may optionally be used in a pre-analytical step if pooling is to be performed.

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 deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[6-8] 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.[9,10] 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

Store at 2-8°C
480 test cassette (P/N 08244049190)

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

Store at 2-8°C
(P/N 08460981190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Babesia Positive Control (Babesia (+) C)	< 0.001% Synthetic (armored) *Babesia* RNA encapsulated in MS2 bacteriophage coat protein, Normal human plasma, *Babesia* 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. P261: Avoid breathing dust/fume/gas/mist/vapours/spray. 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 and wash it before reuse. P501: Dispose of contents/ container to an approved waste disposal plant. 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

Table 3 cobas® NHP Negative Control Kit

Store at 2-8°C
(P/N 07002220190)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning*
Normal Human Plasma Negative Control (NHP-NC)	Normal human plasma, *Babesia* 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 dust/fume/gas/mist/ vapours/spray. 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-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**
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***, 5% (w/v) polydocanol***, 2% (w/v) dithiothreitol***, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. 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. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection. 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. 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

***** 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[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*	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

[a] 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**
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 and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer	07435967001 and 07094361001 or 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**
cobas® 6800 System (Option Moveable)	05524245001 and 06379672001
cobas® 6800 System (Fix)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Option for pipetting and pooling	P/N
cobas® **Synergy** Software Dongle	07788339001
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.[11,12] Only personnel proficient in handling infectious materials and the use of **cobas**® Babesia 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 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® Babesia 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 *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.
- **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**® Babesia 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**® Babesia kits and **cobas omni** 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**® 6800/8800 instruments, follow the instructions in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide to properly clean and decontaminate the surface of instrument(s).

Sample collection, transport and storage

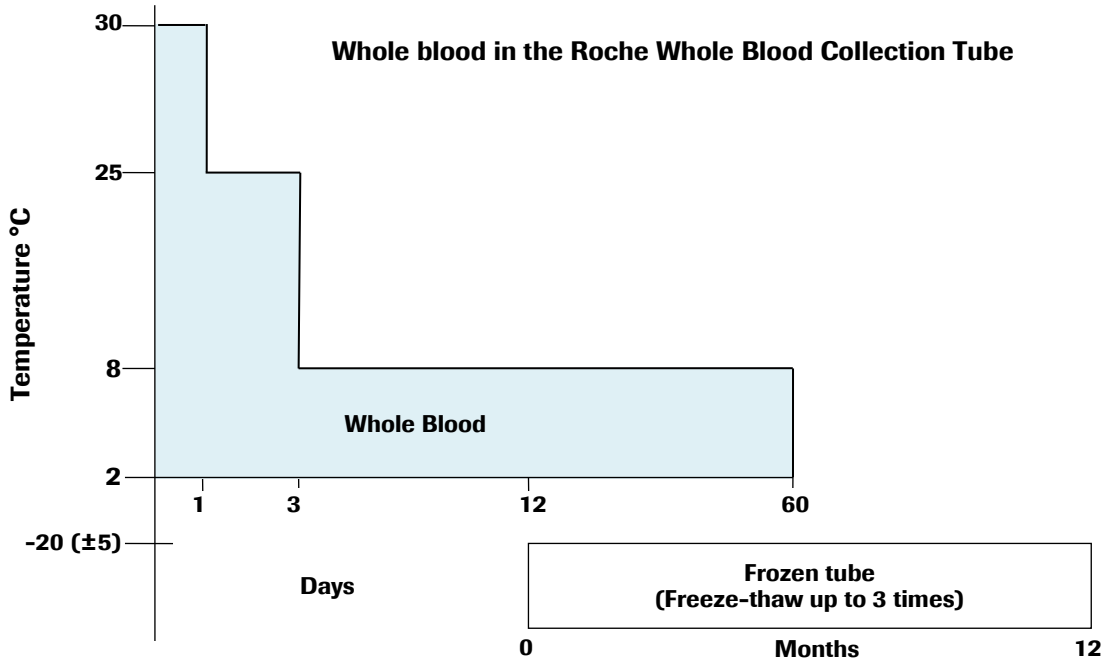
Note: Handle all samples and controls as if they are capable of transmitting infectious agents.
Store all donor samples at specified temperatures.
Sample stability is affected by elevated temperatures.
Centrifuge samples at 1000 rcf (relative centrifugal force) for 2 minutes.

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 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.

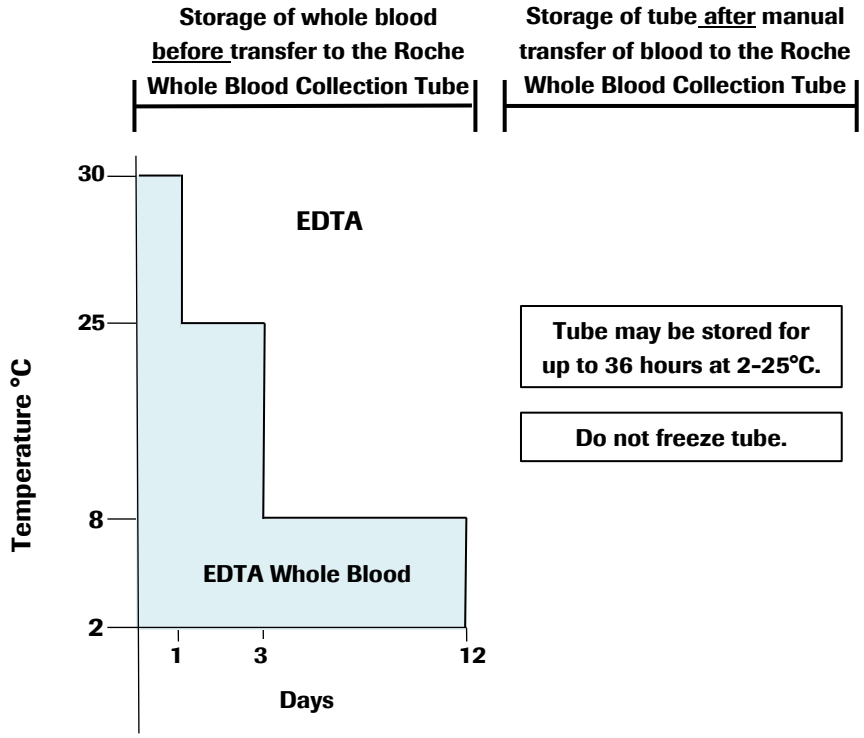
Figure 1 Sample storage conditions for samples collected in the Roche Whole Blood Collection Tube



- 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 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

Automated sample pipetting and pooling (optional)

cobas® **Synergy** Core can be used as an optional component of the **cobas**® 6800/8800 Systems for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample. Refer to the **cobas**® **Synergy** software User Assistance for more information.

Procedural notes

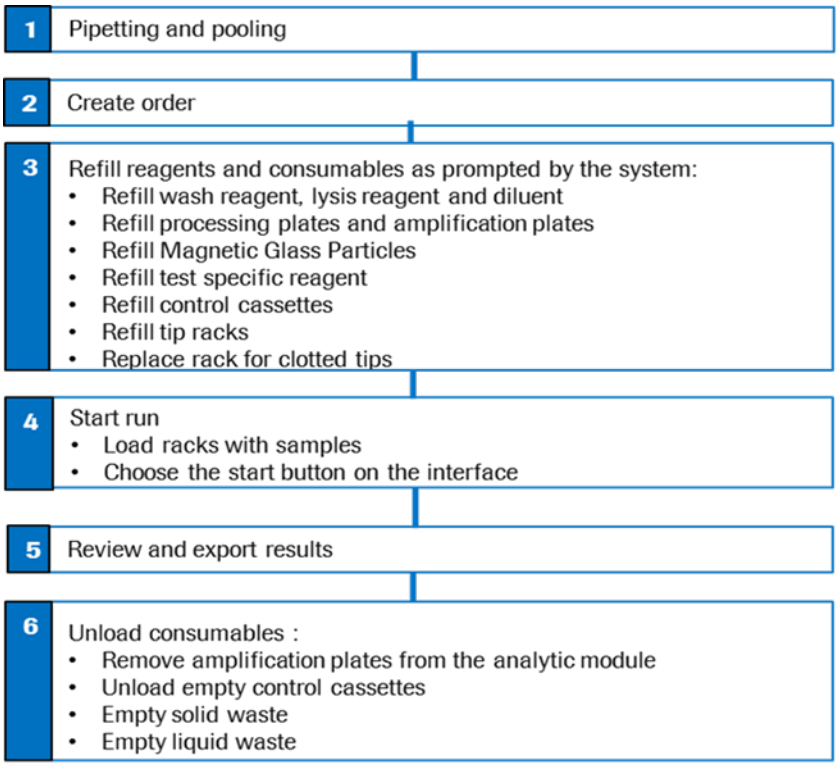
- Do not use **cobas**® Babesia reagents, **cobas**® Babesia Control Kit, **cobas**® NHP Negative Control Kit or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems – User Assistance and/or User Guide or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures for proper maintenance of instruments.

Running cobas® Babesia

The test procedure is described in detail in the **cobas**® 6800/8800 Systems User Assistance and/or User Guide or refer to the **cobas**® **Synergy** software User Assistance and/or User Guide as applicable for details on optional pooling procedures.

Figure 3 below summarizes the procedure.

Figure 3 **cobas**® Babesia procedure



Results

The **cobas**® 6800/8800 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**® 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 both controls.

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**
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**® 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: *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**® 6800/8800 software and should be interpreted as follows:

Table 10 Target results for individual target result interpretation

Target results	Interpretation
Babesia Non-Reactive	No target signal detected for *Babesia* and IC signal detected.
Babesia Reactive	Target signal detected for *Babesia* 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, **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.
- 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
Babesia microti	iRBC/mL	6.1	5.0	7.9
Babesia duncani	iRBC/mL	50.2	44.2	58.8
Babesia divergens	iRBC/mL	26.1	22.3	31.8
Babesia venatorum	iRBC/mL	40.0	34.1	48.7

Table 12 Reactivity rates summary for *Babesia microti*

***Babesia* 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*

***Babesia* 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*

***Babesia* 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*

Babesia **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 3 x 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**
Anaplasma phagocytophilum	Hepatitis B Virus	*Plasmodium falciparum*	*Candida albicans*
Propionibacterium acnes	Hepatitis C Virus	-	-
Staphylococcus aureus	Human Immunodeficiency Virus	-	-
Staphylococcus epidermidis	Parvovirus B19	-	-
Borrelia burgdorferi	West Nile Virus	-	-
Borrelia hermsii	-	-	-
Borrelia parkeri	-	-	-
Borrelia recurrentis	-	-	-

Analytical specificity – interfering substances

Endogenous interference substances

Whole blood samples with abnormally high levels of triglycerides (33 g/L), hemoglobin (≥ 20 g/L), 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**® 6800 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. The 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	Sensitivity (95% CI*) Upper Limit
Neat	609	609	0	100.0%	99.4%	100.0%
1:6	609	609	0	100.0%	99.4%	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 Reactive	cobas® Babesia Result Non-Reactive	Estimate in Percent (95% Exact CI)
Overall	**Clinical Specificity**	168,972	2	168,970	99.999 (99.996, 100.000)
Non Endemic	**Clinical Specificity**	10,824	0	10,824	100.000 (99.966, 100.000)
Low Endemic	**Clinical Specificity**	14,217	0	14,217	100.000 (99.974, 100.000)
High Endemic	**Clinical Specificity**	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* Positive n (%)	Donation Status* Negative n (%)	Total N
Overall, Reactive	9 (100.000)	2 (0.001)	11
Overall, Non-Reactive	0 (0.000)	168,970 (99.999)	168,970
Overall, Total	9	168,972	168,981
Non Endemic, Reactive	1 (100.000)	0 (0.000)	1
Non Endemic, Non-Reactive	0 (0.000)	10,824 (100.000)	10,824
Non Endemic, Total	1	10,824	10,825
Low Endemic, Reactive	0 (0.000)	0 (0.000)	0
Low Endemic, Non-Reactive	0 (0.000)	14,217 (100.000)	14,217
Low Endemic, Total	0	14,217	14,217
High Endemic, Reactive	8 (100.000)	2 (0.001)	10
High Endemic, Non-Reactive	0 (0.000)	143,929 (99.999)	143,929
High Endemic, 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.

Pools of 6 testing results

The clinical specificity for **cobas**® Babesia for donations tested in pools of six (PP6) overall across all endemicity categories was 100% (27,606/27,606; 95% CI: 99.987% to 100%) (Table 21). Specificity results were the same across the 3 endemicity categories (non-, low-, and high-endemic).

Table 21 Clinical specificity of **cobas**® Babesia – donations tested in pools of 6 only (donation level)

Endemicity	Parameter	Total Number of Status-Negative Donations	cobas® Babesia Reactive	cobas Babesia Non-Reactive	Estimate in Percent (95% Exact CI)
Non Endemic	**Clinical Specificity**	6,485	0	6,485	100.000 (99.943, 100.000)
Low Endemic	**Clinical Specificity**	5,834	0	5,834	100.000 (99.937, 100.000)
High Endemic	**Clinical Specificity**	15,287	0	15,287	100.000 (99.976, 100.000)
Overall	**Clinical Specificity**	27,606	0	27,606	100.000 (99.987, 100.000)

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

Table 22 shows the comparison of **cobas**® Babesia results and donation status for 27,729 evaluable donations from which whole blood samples were tested in PP6.

Table 22 Comparison of **cobas**® Babesia results with donation status by endemicity – pools of 6 (donation level)

cobas Babesia Result	Donation Status Positive n (%)	Donation Status Negative n (%)	Total N
Overall, Reactive	7 (100.000)	0 (0.000)	7
Overall, Non-Reactive	0 (0.000)	27,722 (100.000)	27,722
Overall, Total	7	27,722	27,729
Non-Endemic, Reactive	0 (0.000)	0 (0.000)	0
Non-Endemic, Non-Reactive	0 (0.000)	6,590 (100.000)	6,590
Non-Endemic, Total	0	6,590	6,590
Low Endemic, Reactive	0 (0.000)	0 (0.000)	0
Low Endemic, Non-Reactive	0 (0.000)	5,845 (100.000)	5,845
Low Endemic, Total	0	5,845	5,845
High Endemic, Reactive	7 (100.000)	0 (0.000)	7
High Endemic, Non-Reactive	0 (0.000)	15,287 (100.000)	15,287
High Endemic, Total	7	15,287	15,294

Note: Only evaluable donations are included in this summary table. A total of 116/27,729 (0.42%) donations were tested individually (not in pools of 6) in the above table.
Note: 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.

Table 23 summarizes the pool reactivity for the 4,610 qualifying PP6. Out of 4,610 PP6, 4,603 (99.85%) pools were non-reactive and 7 (0.15%) were reactive on **cobas**® Babesia. Out of 7 reactive pools, 7 contained a status-positive donation, and 0 were donation-status negative (false reactive). The overall pool specificity of **cobas**® Babesia was 100% (4,603/4,603 pools; 95% CI: 99.920 to 100%).

Table 23 Pool reactivity in volunteer blood donors

Category	Number of Pools	Percentage of Pools Tested
Total pools tested[a]	4610	100
Non-reactive pools[b]	4603	99.8
Non-reactive pools with all donations status-negative	4603	100
Non-reactive pools with at least one status-positive donation	0	0
Reactive pools[b]	7	0.2
Reactive pools with at least one status-positive donation	7	100
Reactive pools with donations status negative (false-reactive pools)	0	0

[a] Note: 37/4610 pools had < 6 donations.

[b] 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.

For whole blood samples that were tested in PP6, 260 (99.2%) valid cobas Babesia batches yielded 4,748 (99.00%) valid 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 0.5 x, 1-2 x, and approximately 3 x 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 24 (*B. microti*), Table 25 (*B. duncani*), Table 26 (*B. divergens*), and Table 27 (*B. venatorum*)]. This study demonstrated that **cobas**® Babesia for use on the **cobas**® 6800/8800 Systems shows reproducible performance across the variables assessed (lot, site, day, batch, and within batch) for detecting *Babesia*.

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

Babesia microti **Concentration**	**Site ID**	**Site % Reactive Results**	**Lot ID**	**Lot % Reactive Results**	**Day ID**	**Day % Reactive Results**	**Batch ID**	**Batch % Reactive 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)
~0.5 x LoD	2	96.7% (87/90)	2	100% (90/90)	2	94.4% (51/54)	2	96.3% (130/135)
~0.5 x LoD	3	96.7% (87/90)	3	98.9% (89/90)	3	90.7% (49/54)	-	-
~0.5 x LoD	-	-	-	-	4	96.3% (52/54)	-	-
~0.5 x LoD	-	-	-	-	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)
1-2 x LoD	2	100.0% (90/90)	2	100.0% (90/90)	2	100.0% (54/54)	2	100.0% (134/134)
1-2 x LoD	3	100.0% (89/89)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
1-2 x LoD	-	-	-	-	4	100.0% (53/53)	-	-
1-2 x LoD	-	-	-	-	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)
~3 x LoD	2	100.0% (90/90)	2	100.0% (90/90)	2	100.0% (54/54)	2	100.0% (135/135)
~3 x LoD	3	100.0% (90/90)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
~3 x LoD	-	-	-	-	4	100.0% (54/54)	-	-
~3 x LoD	-	-	-	-	5	100.0% (54/54)	-	-

Note: LoD = Limit of detection.

Table 25 Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia duncani*

Babesia duncani **Concentration**	**Site ID**	**Site % Reactive Results**	**Lot ID**	**Lot % Reactive Results**	**Day ID**	**Day % Reactive Results**	**Batch ID**	**Batch % Reactive 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)
~0.5 x LoD	2	68.9% (62/90)	2	54.4% (49/90)	2	63.0% (34/54)	2	61.5% (83/135)
~0.5 x LoD	3	74.4% (67/90)	3	73.3% (66/90)	3	57.4% (31/54)	-	-
~0.5 x LoD	-	-	-	-	4	64.8% (35/54)	-	-
~0.5 x LoD	-	-	-	-	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)
1-2 x LoD	2	100.0% (90/90)	2	100.0% (89/89)	2	100.0% (54/54)	2	100.0% (135/135)
1-2 x LoD	3	100.0% (89/89)	3	100.0% (90/90)	3	100.0% (54/54)	-	-
1-2 x LoD	-	-	-	-	4	100.0% (54/54)	-	-
1-2 x LoD	-	-	-	-	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)
~3 x LoD	2	100.0% (89/89)	2	100.0% (90/90)	2	100.0% (54/54)	2	100.0% (134/134)
~3 x LoD	3	100.0% (89/89)	3	100.0% (89/89)	3	100.0% (53/53)	-	-
~3 x LoD	-	-	-	-	4	100.0% (54/54)	-	-
~3 x LoD	-	-	-	-	5	100.0% (53/53)	-	-

Note: LoD = Limit of detection.

Table 26 Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia divergens*

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

Note: LoD = Limit of detection.

Table 27 Test results summarized by site, lot, day, and batch (positive panel members) – *Babesia venatorum*

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

Note: LoD = Limit of detection.

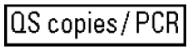
Additional information

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
Test duration	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 *in vitro* diagnostic medical devices.
	US Only: Federal law restricts this device to sale by or on the order of a physician.		Date of manufacture		
	*In Vitro* diagnostic medical device		Do not reuse		

US Customer Technical Support 1-800-526-1247

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References

1. Vannier EG, Diuk-Wasser MA, Ben Mamoun C, Krause PJ. Babesiosis. *Infect Dis Clin North Am*. 2015;29:357-70. PMID: 25999229.
2. Herwaldt BL, Linden JV, Bosserman E, et al. Transfusion-associated babesiosis in the United States: a description of cases. *Ann Intern Med*. 2011;155:509-19. PMID: 21893613.
3. U.S. Centers for Disease Control and Prevention. Babesiosis surveillance - 18 States, 2011. *MMWR Morb Mortal Wkly Rep*. 2012;61:505-9. PMID: 22785341.
4. Leiby DA. Transfusion-Associated Babesiosis: Shouldn't We Be Ticked Off? *Ann Intern Med*. 2011;155:556-7.
5. Moritz ED, Winton CS, Tonnetti L, et al. Screening for Babesia microti in the U.S. Blood Supply. *N Engl J Med*. 2016;375:2236-45. PMID: 27959685.
6. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. *Gene*. 1990;93:125-8. PMID: 2227421.
7. Savva R, McAuley-Hecht K, Brown T, Pearl L. The structural basis of specific base-excision repair by uracil-DNA glycosylase. *Nature*. 1995;373:487-93. PMID: 7845459.
8. Mol CD, Arvai AS, Slupphaug G, et al. Crystal structure and mutational analysis of human uracil-DNA glycosylase: structural basis for specificity and catalysis. *Cell*. 1995;80:869-78. PMID: 7697717.
9. Higuchi R, Dollinger G, Walsh PS, Griffith R. Simultaneous amplification and detection of specific DNA sequences. *Biotechnology (N Y)*. 1992;10:413-7. PMID: 1368485.
10. Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res*. 1996;6:986-94. PMID: 8908518.
11. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health HHS Publication No. (CDC) 21-1112, revised December 2009.
12. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4:Wayne, PA;CLSI, 2014.

Document revision

Document Revision Information	
Doc Rev. 1.0 09/2019	First Publishing.
Doc Rev. X.0 XX/2020	Added the pooling claim to the following sections: · **Intended use** · **Principles of the procedure** · **Table 8** · **Figure 3** · **Instructions for use** · **Clinical performance evaluation of additional clinical studies conducted** Increased the storage to 12 months at -20°C in the **Sample collection, transport and storage** section. Please contact your local Roche Representative if you have any questions.