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Procleix® Babesia Assay

For *In Vitro* Diagnostic Use

IVD

Rx Only
5000 Test Kit

CONTENTS

INTENDED USE

The Procleix® Babesia Assay is a qualitative in vitro nucleic acid amplification test for the detection of RNA from *Babesia* species (*B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*) in whole blood specimens. It is intended for use in screening individual human donors, including donors of whole blood and blood components, and in screening living donors of organ and tissue. It is also intended for use in screening cadaveric (non-heart-beating) donors for *B. microti*. It is not intended for use on cord blood specimens. Lysed individual donor whole blood samples are tested either individually or in pools of equal aliquots of not more than 16. Cadaveric donor specimens must be tested individually and not pooled.

This assay is not intended for use as an aid in diagnosis of *Babesia* infection.

SUMMARY AND EXPLANATION OF THE TEST

Babesiosis is a disease caused by a group of tick-borne, intraerythrocytic protozoan parasites in the genus *Babesia*. It is most often transmitted to humans through the bite of an infected nymphal tick from the Ixodidae family.[1] In addition to vector-borne transmission, the parasite can be transmitted through transplacental and perinatal routes[2] and from contaminated blood products.[3] To date there are four main *Babesia* species that have been confirmed to infect humans: *B. microti, B. divergens, B. duncani* and *B. venatorum*.[4]

B. microti is one of the most commonly transmitted pathogens by blood transfusion in the US.[5] Although *Babesia* often causes an asymptomatic infection in immunocompetent adults, some people can develop flu-like symptoms such as fever, chills, sweats, headache, body aches, loss of appetite, nausea, or fatigue. For the at-risk recipient population, including neonates, the elderly, individuals that are immunosuppressed, asplenic or with kidney or liver disease, babesiosis can cause severe complications and can be fatal.[6] Associated mortality with transfusion-transmitted babesiosis (TTB) in the at- risk population is estimated at about 20%.[7]

The first identified *B. microti* case in the United States was in 1966,[8] and now *B. microti* is recognized to be endemic in the upper Midwest and Northeastern states.[9] In the Pacific Northwest region, babesiosis cases caused by *B. duncani* have been reported.[10-12] Isolated but severe cases of *B. divergens* infections have been reported in Missouri, Kentucky and Washington States.[13-15] In Europe, *B. divergens*, is the main species responsible for babesiosis. The vast majority of European cases have been reported in the British Isles, France and Portugal, along with some cases attributed to *B. microti* and *B. venatorum* in Germany, Poland, Austria and Italy. Additional cases have been reported in Asia, Australia, Africa and South America.[4, 6] Cases of human babesiosis have been reported throughout the world and Babesia is now becoming recognized as a significant human pathogen.

The Procleix Babesia Assay, which detects the 18S ribosomal RNA of *B. microti, B. divergens, B. duncani*, and *B. venatorum,* uses the same target capture, transcription-mediated nucleic acid amplification (TMA) technology as other FDA licensed Procleix blood screening assays.

PRINCIPLES OF THE PROCEDURE

The Procleix Babesia Assay is performed on the fully automated Procleix Panther System.

The Procleix Babesia Assay requires a whole blood sample lysis step that can be performed manually or on the Procleix Xpress System. During sample lysis on the Procleix Xpress System, whole blood is added to Parasite Transport Medium (PTM) allowing the rupture of the red blood cell membranes, as well as the release of the parasites and the liberation and stabilization of the RNA into the lysis solution. The Procleix Xpress System pipettes 2.7 mL of PTM into empty individual lysate tubes. Following the prefilling of the lysate tubes with PTM, whole blood sample tubes are loaded on to the worktable. The pipettor mixes the whole blood sample and then 0.9 mL of whole blood is transferred to the lysate tube containing 2.7 mL of PTM, followed by a lysate mixing step. Lysate (0.3 mL) can be transferred to a master pool tube to create a pool of 8 or 16 donations as initiated by the operator at the beginning of the run. Upon completion of lysis, individual donor lysate tubes and/or master pool tubes are loaded on to the Procleix Panther System. The Procleix Xpress System also provides an option for preparing individual donor lysate tubes. For manual lysis, follow the same volumes and steps as the automated lysis and pooled procedures.

Once the lysates are made, the Procleix® Babesia Assay involves three main steps, which take place in a single tube on the Procleix Panther System: target capture, *Babesia* RNA target amplification by TMA,[16] and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA).[17]

During sample preparation, RNA is isolated from the lysate via target capture. Oligonucleotides (capture oligonucleotides) that are homologous to highly conserved regions of *Babesia* RNA are hybridized to the *Babesia* RNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. The T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The Procleix Babesia Assay utilizes the TMA method to amplify regions of *Babesia* RNA.

Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured by a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control, and assay calibrator via the working Target Capture Reagent. The Internal Control in the Procleix Babesia Assay controls for specimen processing, amplification, and detection steps. Internal Control signal is discriminated from the *Babesia* signal by the differential kinetics of light emission from probes with different labels.[17] Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to *Babesia* is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels.[18]

The Procleix Babesia Assay Calibrators are used to determine the analyte and internal control cutoff values and establish run validity.

REAGENTS

Procleix Babesia Assay Reagents

Internal Control Reagent
A HEPES buffered solution containing detergent and an RNA transcript.
*Store **unopened reagent** at –35° to –15°C.*

Target Capture Reagent
A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles.
***Note:** Internal Control Reagent must be added to Target Capture Reagent before use in the assay.*
Store at 2° to 8°C. (Do not freeze)

Amplification Reagent
Primers, dNTPs, NTPs, and cofactors in TRIS buffered solution containing ProClin® 300 preservative.
*Store **unopened reagent** at –35° to –15°C.*

Enzyme Reagent
MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.
*Store **unopened reagent** at –35° to –15°C.*

Probe Reagent
Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.
*Store **unopened reagent** at –35° to –15°C.*
⚠ The Probe Reagent is light-sensitive. Protect from light during storage.

Selection Reagent
Borate buffered solution containing surfactant.
Store at 15° to 30°C.

Procleix Babesia Assay Calibrators

C

Procleix Babesia Assay Negative Calibrator
A HEPES buffered solution containing detergent.
Store at –35° to –15°C.

C

Procleix Babesia Assay Positive Calibrator
A HEPES buffered solution containing detergent and a Babesia RNA transcript.
Store at –35° to –15°C.

Procleix Panther System Reagents

R1

Auto Detect 1
Aqueous solution containing hydrogen peroxide and nitric acid.
*Store **unopened reagent** at 15° to 30°C.*

R2

Auto Detect 2
1.6 N sodium hydroxide.
*Store **unopened reagent** at 15° to 30°C.*

W

Wash Solution
HEPES buffered solution.
*Store **unopened reagent** at 15° to 30°C.*

O

Oil
Silicone oil.
*Store **unopened reagent** at 15° to 30°C.*

DF

Buffer for Deactivation Fluid
Sodium bicarbonate buffered solution.
*Store **unopened reagent** at 15° to 30°C.*

Additional Reagent

Parasite Transport Medium (PTM)
A TRIS buffered solution containing detergent
Store at 15° to 30°C.

STORAGE AND HANDLING INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. ⚠ The Probe Reagent is light-sensitive. Protect this reagent from light during storage.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other Procleix assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

Note: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

F. Do not refreeze Internal Control, Amplification, Enzyme, and Probe Reagents after the initial thaw.

G. Calibrators are single use vials and must be discarded after use.

H. To reduce the possibility of reagent cross-contamination, it is recommended to use new reagent caps when unloading reagent bottles from the Panther instrument and storing them.

I. If precipitate forms in the Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under REAGENT PREPARATION.

J. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness are indicative of microbial contamination), they should not be used.

K. For instructions on preparation of reagents, see instructions under REAGENT PREPARATION and the Procleix RPI 250 Operator's Manual or the Procleix Reagent Equilibration System Operator's Manual.

L. Consult the following table for storage information.

Reagent/Fluids	**Unopened Reagent**	**Opened Reagent (Opened/Thawed Stability)***		
	Storage Temperature	**Room Temperature**	**Onboard Stability**	**Storage Temperature**
Internal Control Reagent (IC)	–35° to –15°C	Up to 8 hours at RT prior to combining with TCR		
Target Capture Reagent (TCR)	2° to 8°C	20 hours		
working Target Capture Reagent (wTCR)		72 hours	60 hours	30 days at 2° to 8°C
Amplification Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Enzyme Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Probe Reagent	–35° to –15°C	72 hours	60 hours	30 days at 2° to 8°C
Selection Reagent	RT	30 days	60 hours	30 days at RT
Calibrators	–35° to –15°C	8 hours, single-use reagent		
Parasite Transport Medium (PTM)	RT	90 days**		90 days at RT
Auto Detect Reagents	RT	60 days at RT		
Buffer for Deactivation Fluid	RT	60 days at RT		
Oil	RT	60 days at RT		
Wash Solution	RT	60 days at RT		

RT = Room Temperature

RT stability includes onboard stability time on the Procleix Panther System.

- The RT stability period starts as soon as the reagents are removed from the RPI 250 or RES after the preparation program is completed.
- If opened reagents are placed in the RPI 250 or RES at the room temperature program, the time duration is included in the total RT stability.
- The RT stability time must occur within 30 days, which includes onboard stability. See REAGENT PREPARATION, Item C for more information.

* If using Panther System Software version 7.2 and higher:

- RT stability (wTCR and Amplification, Enzyme, and Probe Reagents) is 84 hours.
- Onboard stability (wTCR and Amplification, Enzyme, Probe, and Selection Reagents) is 72 hours.

If using RPI 250 File 3 or RES Room Temperature program for thawing unopened reagents (TCR and Amplification, Enzyme, and Probe Reagents), reagents must remain in the RPI 250 or RES for 4 to 20 hours. Refer to the *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Reagent Equilibration System Operator's Manual* for additional information.

Caution: ⚠ Only the reagent onboard stability is tracked by the Procleix Panther System software. The time reagents remain at RT when not onboard the Procleix Panther System MUST be manually tracked by the user to ensure maximum allotted RT time is not exceeded.
Maintain reagents at the appropriate storage condition when not in use. Return reagents to their appropriate storage conditions without delay unless they are on the RPI 250, the RES, or the Procleix Panther System. Deviations from storage may impact the performance of the assay.

**Pre-filled tubes with PTM can be stored for up to 48 hours at RT.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Warning: Handle all specimens as if they are potentially infectious agents.

Note: Take care to avoid cross-contamination during the sample handling steps. For example, discard used material without passing over open tubes.

LIVING DONOR WHOLE BLOOD SPECIMENS

A. Only blood specimens collected in plastic tubes may be used.

B. Whole blood collected in K_2EDTA, K_3EDTA, Sodium Citrate and CPDA may be used. Follow sample tube manufacturer's instructions.

Whole blood from individual donor specimens may be stored for a total of 13 days from the time of collection to the time of testing with the following conditions:

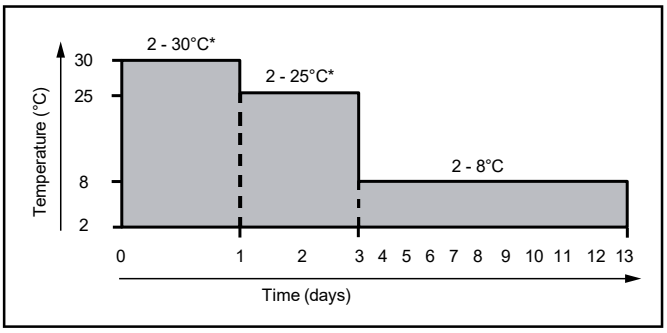
For storage above 8°C, specimens may be stored for 72 hours up to 25°C, and up to 30°C for any 24 hour period during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature chart below (Figure 1: Living Donor Whole Blood Specimen Stability).

Stability in frozen/thawed whole blood has not been evaluated.

Figure 1: Living Donor Whole Blood Specimen Stability



***The cumulative time spent at the elevated temperatures cannot exceed these limits .**

C. Individual donor lysates may be stored for a total of 7 days from the time of the lysis to the time of testing under the following conditions:

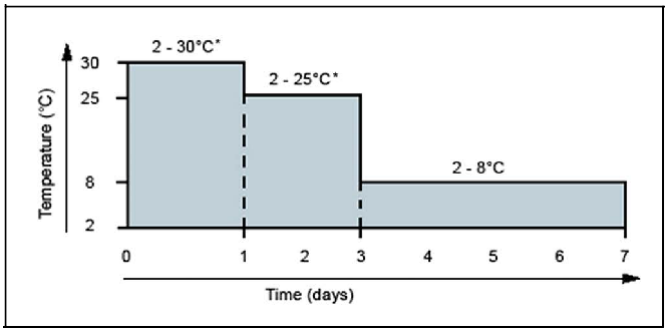
For storage above 8°C, individual donor lysates may be stored for 72 hours up to 25°C, and up to 30°C for any 24 hour period during the 72 hours.

Other than noted above, specimens are stored at 2° to 8°C.

In addition, individual donor lysates may be stored for up to 1 year at ≤ -20°C before testing.

Refer to the example storage temperature chart below (Figure 2: Living Donor Individual Donor Lysate Stability).

Figure 2: Living Donor Individual Donor Lysate Stability



***The cumulative time spent at the elevated temperatures cannot exceed these limits.**

D. Pooled donor lysates may be stored for a total of 7 days from the time of the lysis to the time of testing under the following conditions:

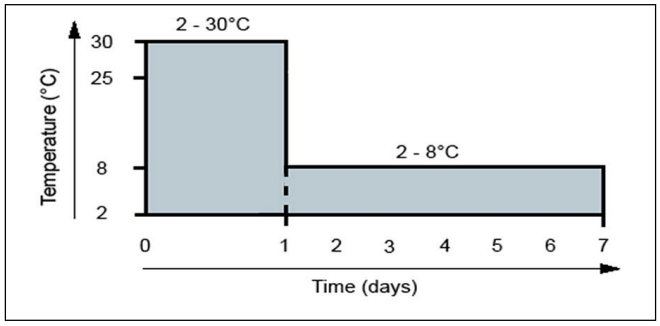
For storage above 8°C, pooled donor lysates may be stored for any 24 hour period up to 30°C.

Other than noted above, specimens are stored at 2° to 8°C.

Freezing pooled donor lysates may affect sensitivity.

Refer to the example storage temperature chart below (Figure 3: Living Donor Pooled Donor Lysate Stability).

Figure 3: Living Donor Pooled Donor Lysate Stability



***The cumulative time spent at the elevated temperatures cannot exceed these limits**

E. Specimen Stability may be affected by elevated temperature.

F. No adverse effect on assay performance was observed when individual donor lysates were subjected to 3 freeze-thaw cycles. Pooled donor lysates should not be frozen

Note: When testing frozen lysates, allow specimens to reach room temperature prior to processing by following these steps:

1. Warm the frozen lysate specimens to a temperature between 28°C and 30°C. This can be achieved by using either a water bath or a dry incubator.

- If using a water bath, place the specimens in a 30°C water bath for at least 30 minutes and no more than 2 hours.
- Alternatively, warm the specimens in a dry incubator set to 28°C - 30°C for at least 1 hour and no more than 2 hours.

2. Mix and homogenize

Every 10 minutes during incubation, remove the specimens from the water bath or dry incubator. Gently invert them 10 times to mix and homogenize the contents. Check for any precipitates. Make sure they are completely dissolved before proceeding.

3. Final Homogenization

Prior to loading on the Panther, gently invert the sample 10 more times to ensure complete homogenization.

G. Ensure that all whole blood samples are at room temperature before loading onto the Procleix Xpress Pipettor.

H. Ensure that all whole blood samples have been mixed preferably on a tube mixer for at least 5 minutes or by inversions for at least 15 times immediately before loading onto the Procleix Xpress Pipettor. Ensure that the whole blood is mixed gently and thoroughly and is homogenous.

I. If testing previously centrifuged samples, or blood with visibly separated plasma and red blood cells, ensure that they are thoroughly mixed until they are homogenous prior to performing the run.

Note: A higher rate of pipetting errors or an increased risk of False Negatives may be encountered when using non-homogenous or previously centrifuged samples.

J. Individual donor lysates or pooled donor lysates should be mixed by inversion if stored for extended periods of time prior to retesting.

K. Do not test more than 4 replicates from an individual donor lysate tube.

L. Other collection and storage conditions should be validated by the user. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

M. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.

N. Whole blood lysate specimen from donors of whole blood, blood components, and other living donors, may be pooled.

O. A Grifols validated pooling process may be used to perform pooling operations that combine aliquots from individual specimens into a single Master Pool Tube, which may be used for further testing. Refer to OTHER MATERIALS AVAILABLE FOR USE WITH PROCLEIX BABESIA ASSAY for Grifols validated pooling solutions.

CADAVERIC DONOR WHOLE BLOOD SPECIMENS

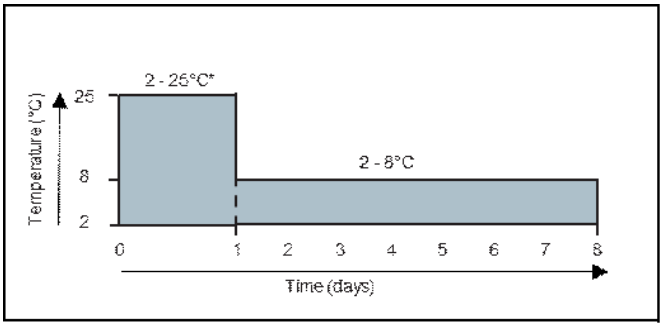
A. Cadaveric blood specimens can be collected in K_2EDTA anticoagulant tubes. Follow sample tube manufacturer's instructions.

B. Specimens should be collected within 24 hours of death if the cadaver was refrigerated (1° to 10°C) within 12 hours of death or according to general standards and/or regulations.

Whole blood from individual cadaveric donor specimens may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

For storage above 8°C, cadaveric specimens may be stored at any time for any 24 hour period up to 25°C. Stability in frozen/thawed cadaveric whole blood has not been evaluated.
Refer to the example storage temperature chart below (Figure 4: Cadaveric Donor Whole Blood Specimen Stability).

Figure 4: Cadaveric Donor Whole Blood Specimen Stability

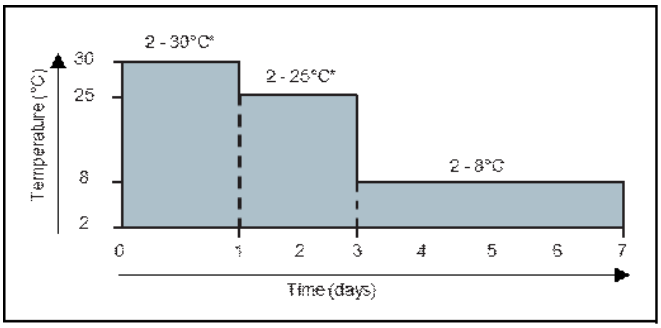


*The cumulative time spent at the elevated temperatures cannot exceed these limits.

C. Cadaveric individual donor lysates may be stored for a total of 7 days from the time of the lysis to the time of testing under the following conditions:

For storage above 8°C, cadaveric individual donor lysates may be stored at any time for 72 hours up to 25°C, and up to 30°C for any 24 hour period during the 72 hours.
Other than noted above, specimens are stored at 2° to 8°C.
In addition, individual donor lysates may be stored for up to 1 year at ≤-20°C before testing.
Refer to the example storage temperature chart below (Figure 5: Cadaveric Individual Donor Lysate Stability).

Figure 5: Cadaveric Individual Donor Lysate Stability



*** The cumulative time spent at the elevated temperatures cannot exceed these limit.**

D. No adverse effect on assay performance was observed when cadaveric individual donor lysates were subjected to 3 freeze-thaw cycles.

Note: When testing frozen lysates, allow specimens to reach room temperature prior to processing by following these steps:

1. Warm the frozen lysate specimens to a temperature between 28°C and 30°C. This can be achieved by using either a water bath or a dry incubator.

- If using a water bath, place the specimens in a 30°C water bath for at least 30 minutes and no more than 2 hours.
- Alternatively, warm the specimens in a dry incubator set to 28°C - 30°C for at least 1 hour and no more than 2 hours.

2. Mix and homogenize

Every 10 minutes during incubation, remove the specimens from the water bath or dry incubator. Gently invert them 10 times to mix and homogenize the contents. Check for any precipitates. Make sure they are completely dissolved before proceeding.

3. Final Homogenization

Prior to loading on the Panther, gently invert the sample 10 more times to ensure complete homogenization.

E. Specimen stability may be affected by elevated temperature.

F. Whole blood with visibly separated plasma and red blood cells or previously spun samples should be mixed with inverting the samples at least 15 times or until the whole blood is homogeneous.

G. Cadaveric individual donor lysates should be mixed by inversion if stored for extended periods of time prior to retesting.

H. Do not test more than 4 replicates from an individual donor lysate tube.

I. Other cadaveric blood specimen collection, handling, and storage conditions must be validated by the user. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable international regulations covering the transport of clinical specimens and etiologic agents.

J. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen and handling processing.

K. Cadaveric individual donor lysates may be diluted to overcome potential sample inhibitory substances. Lysate may be diluted 1:4 in PTM (i.e., 0.3 mL lysate plus 1.2 mL PTM). Diluted specimens should be inverted several times to mix and then may be used in the standard assay procedure.

L. Studies to validate these conditions were performed on negative cadaveric specimens spiked with *B. microti* parasite only.

M. Lysates from cadaveric specimens should not be pooled.

MATERIALS REQUIRED

Component	Part Number/Quantity	
Procleix Babesia Assay Kit	**9051246** (5000 Test Kit)	
Internal Control Reagent	20 x 2.8 mL	
Amplification Reagent	20 x 26 mL	
Enzyme Reagent	20 x 13.4 mL	
Probe Reagent	20 x 34.7 mL	
Target Capture Reagent	20 x 161 mL	
Selection Reagent	20 x 91 mL	
Procleix Babesia Assay Calibrators Kit	**9051253** (75 Sets)	
Negative Calibrator	75 x 2.2 mL	
Positive Calibrator	75 x 2.2 mL	
Procleix Assay Fluids Kit	**303344**	
Wash Solution	1 x 2.9 L	
Oil	1 x 260 mL	
Buffer for Deactivation Fluid	1 x 1.4 L	
Component	**Part Number/Quantity**	**Part Number/Quantity**
Procleix Auto Detect Reagents Kit	**303345**	**9053575**
Auto Detect 1	1 x 245 mL	4 x 245 mL
Auto Detect 2	1 x 245 mL	4 x 245 mL
Procleix Parasite Transport Medium (PTM)	**9051577**	
	1 x 1.6 L	

Disposables *(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)*	Quantity	Part Number
Multi-Tube Units (MTUs)	1 case of 100	**104772**
Waste Bag Kit	1 box of 10	**902731**
MTU Waste Cover	1 box of 10	**504405**
Reagent Spare Caps (TCR and Selection Reagents)	1 bag of 100	**CL0039**
Reagent Spare Caps (Amplification and Probe Reagents)	1 bag of 100	**CL0042**
Reagent Spare Caps (Enzyme Reagents)	1 bag of 100	**501619**

Equipment

Procleix Panther System and operator's manual
Procleix Reagent Preparation Incubator 250 (RPI 250) with Independent Temperature Monitor (ITM) and operator's manual or
Procleix Reagent Equilibration System (RES) and operator's manual

Other

Advanced Cleaning Solution	1 bottle (255 mL)	**PRD-04550**

OTHER MATERIALS AVAILABLE FOR USE WITH PROCLEIX BABESIA ASSAY

General Equipment/Software

Procleix Xpress System, *Procleix Xpress System Operator's Manual*, and Procleix NAT Manager software
For instrument specifics and ordering information, contact Grifols Technical Service.

MATERIALS REQUIRED BUT NOT PROVIDED

Bleach (for use in final concentrations of 5 to 8.25% sodium hypochlorite and 0.5 to 0.7% sodium hypochlorite)
Alcohol (70% ethanol, 70% isopropyl alcohol solution, or 70% isopropyl alcohol wipes)
Disposable 1000 µL conductive filter tips in rack approved for use with the Procleix Panther System. Contact Grifols Technical Service for approved tips.
For Manual lysis:
Lysate tubes or 12–16 x 75 mm polypropylene plastic or siliconized glass tubes
50 mL graduated conical tube for decanting Parasite Transport Medium (PTM)

Calibrated pipettes and 1000 µL pipette tips with filter

PRECAUTIONS

A. For *in vitro* diagnostic use.

B. When performing testing with different Procleix Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing. In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. Specimens may be infectious. Use Universal Precautions when performing the assay. Proper handling and disposal methods should be established according to local, state, and federal regulations. Only personnel adequately qualified and proficient in the use of the Procleix Babesia Assay and trained in handling infectious materials should perform this procedure.

D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. The Enzyme Reagent contains sodium azide as a preservative. Do not use metal tubing for reagent transfer. If solutions containing azide compounds are disposed of in a plumbing system, they should be diluted and flushed with generous amounts of running water. These precautions are recommended to avoid accumulation of deposits in metal piping in which explosive conditions could develop.

F. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Babesia Assay and the *Procleix Panther System Operator's Manual* prior to performing an assay run.

G. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes, and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry, and follow appropriate site procedures.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state, and federal regulations. Thoroughly clean and disinfect all work surfaces.

I. Use only specified disposables.

J. Do not use kit after expiration date.

K. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

L. Avoid microbial and nuclease contamination of reagents.

M. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE AND HANDLING INSTRUCTIONS and REAGENT PREPARATION.

N. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE, AND HANDLING for specific instructions.

O. To reduce the risk of invalid results when testing frozen lysates, ensure specimens are brought to room temperature. See SPECIMEN COLLECTION, STORAGE, AND HANDLING for specific instructions.

P. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present. See REAGENT PREPARATION for specific instructions.

Q. Do not combine any assay reagents or fluids without specific instruction. Do not top off reagent or fluids. The Procleix Panther System verifies reagent levels.

R. Only the reagent onboard stability is tracked by the Procleix Panther System Software. The time reagents remain at room temperature when not onboard the Procleix Panther System MUST be tracked by the user to ensure maximum allotted room temperature time is not exceeded.

S. Some reagents of this kit are labeled with risk and safety symbols and should be handled accordingly. Safety Data Sheets are accessible from the manufacturer's website.

Procleix Selection Reagent
 *Boric Acid 3.63 Weight-%* **WARNING** Harmful if inhaled Avoid breathing dust/fume/gas/mist/vapors/spray Use only outdoors or in a well-ventilated area IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing Call a POISON CENTER or doctor/physician if you feel unwell

Procleix Auto Detect 2

Sodium Hydroxide 6.04 Weight-%
DANGER

Causes severe skin burns and eye damage

Do not breathe dust/fume/gas/mist/vapors/spray
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection

Immediately call a POISON CENTER or doctor/physician
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 or doctor/physician
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
Wash contaminated clothing before reuse
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Immediately call a POISON CENTER or doctor/physician
IF SWALLOWED: rinse mouth. DO NOT induce vomiting

Store locked up
Dispose of contents/container to an approved waste disposal plant

Procleix Buffer for Deactivation Fluid

Sodium Hydroxide 1–3 Weight-%
Sodium Hypochlorite <1 Weight-%
WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation

P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P362 - Take off contaminated clothing and wash before reuse
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention

T. Some reagents of this kit are labeled with hazard “H” and precaution “P” codes per international regulations and should be handled accordingly. Safety Data Sheets are accessible from the manufacturer’s website.

U. The Procleix Panther System groups a kit of reagents into a matched set the first time that it scans their barcodes during the inventory process and are required to be run as a set each subsequent time that they are loaded onto the Procleix Panther System. Bottles belonging to a matched set cannot be swapped with bottles in other matched sets of reagents. Refer to the *Procleix Panther System Operator’s Manual* for more information.

V. Refer to additional precautions in the *Procleix Panther System Operator's Manual*.

W. DO NOT heat the Probe Reagent above 35°C when using the RPI 250 or RES. Refer to the *Procleix Reagent Preparation Incubator 250 Operator’s Manual* or the *Procleix Reagent Equilibration System Operator’s Manual*, as applicable.

X. Each Calibrator is designed to be run in triplicate, and excess material in each vial is to be appropriately discarded.

REAGENT PREPARATION

A. Room temperature is defined as 15° to 30°C.

B. Choose a new or opened matched set of reagents. An open set of reagents must be used on either the same Procleix Panther System as used previously or a Procleix Panther System that is connected to that system via Data Sharing.

C. Verify that the reagents have not exceeded the expiration date and/or storage stability times, including onboard stability. Refer to STORAGE AND HANDLING INSTRUCTIONS.

1. The Procleix Panther System tracks the number of hours each reagent and fluid is loaded onboard the analyzer. The Procleix Panther System will not start pipetting specimens if reagents have expired or exceeded their onboard stability. Consult the following table for onboard stability information.

Reagent/Fluid	Onboard Stability
wTCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, Selection Reagent	60 hours*
Wash Solution, Oil, Buffer for Deactivation Fluid, Auto Detect Reagents	60 days

*If using Panther System Software version 7.2 and higher, onboard stability is 72 hours.

D. Remove a bottle of Selection Reagent from room temperature storage.

Note: The Selection Reagent must be at room temperature before use.

1. Selection Reagent may form precipitate if it is inadvertently stored at 2° to 8°C or if the room temperature falls between 2° to 15°C.
2. If cloudiness or precipitate is present, perform Selection Reagent recovery as described in the *Procleix Reagent Preparation Incubator 250 Operator's Manual* or the *Procleix Reagent Equilibration System Operator's Manual*. Do not use if precipitate or cloudiness persists.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
4. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. Refer to the *Procleix Reagent Preparation Incubator 250 Operator's Manual* if using the RPI 250, or the *Procleix Reagent Equilibration System Operator's Manual* if using the RES, to prepare the following reagents: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent.

Note: If precipitate is still present after thawing, Probe Reagent can be incubated with RPI 250 File 3 (room temperature) or RES Room Temperature program to facilitate complete dissolution of precipitate. The Probe Reagent may also be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the water bath should not exceed 30°C. If thawing is conducted on the lab bench, probe reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate.

F. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate, or cloudiness is present (refer to instructions in steps G.4, H, and I below).

Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

G. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI 250 or RES, and refer to the applicable *Procleix Reagent Preparation Incubator 250 Operator's Manual* or *Procleix Reagent Equilibration System Operator's Manual* for instructions.

   Note: If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2° to 8°C storage for subsequent use.
3. Thaw one vial of Internal Control (IC) Reagent up to 24 hours at 2° to 8°C or up to 8 hours at room temperature. Do not use the RPI 250 or RES to thaw Internal Control Reagent.
4. Mix the Internal Control Reagent thoroughly by gentle manual inversion or mechanical inversion using a laboratory rocker.

   Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the Internal Control Reagent at 25° to 30°C in a water bath. Periodically remove Internal Control Reagent from water bath to gently invert until gel is dissolved.
5. Unload TCR from the RPI 250 or RES and warm the Internal Control Reagent to room temperature.
6. Mix thoroughly.
7. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR).
8. Record the date Internal Control Reagent was added, wTCR expiration date (date Internal Control Reagent was added plus 30 days), and lot number used (IC LOT), in the space indicated on the TCR bottle.
9. Retain the IC vial to scan the barcode label into the system.

H. Thaw calibrators at room temperature. **Do not use the RPI 250 or RES to thaw Procleix Babesia Assay Calibrators.**

Note: These are single-use vials which must be thawed prior to each run.

1. Mix calibrators gently by inversion to avoid foaming.
2. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

Note: If gelling occurs, gel must be dissolved prior to use and within the 8 hour thaw period at room temperature. To expedite the dissolution of gel, warm the calibrators at 25° to 30°C in a water bath. Periodically remove calibrators from water bath to gently invert until gel is dissolved.

I. Record the date Wash Solution, Buffer for Deactivation Fluid, Oil, Auto Detect 1, and Auto Detect 2 were first opened and loaded onto the Procleix Panther System (OPEN DATE) in the space provided on the label.

SPECIMEN PREPARATION

AUTOMATED LYSIS INSTRUCTIONS

Refer to the *Procleix Xpress System Operator's Manual* for instructions to prepare samples using the Procleix Xpress System

MANUAL LYSIS INSTRUCTIONS

A. Work Area Preparation

1. Wipe down bench top and pipettes with freshly made 0.5-0.7% sodium hypochlorite and leave in contact with the surface for 15 minutes. Do not allow the sodium hypochlorite solution to dry.
2. Remove sodium hypochlorite by wiping down the bench with deionized water.
3. Cover the work area with a plastic-backed absorbent laboratory bench cover.

B. Manual Lysis Preparation

1. Prepare the number of lysate tubes required (1 per blood sample for individual donor testing) and label as per laboratory protocol.
2. Decant the approximate volume of Parasite Transport Media (PTM) required into the conical tube.
3. Using a single pipette tip, retrieve 2.7 mL of PTM from the conical tube and dispense into each pre-labelled lysis tube.
4. When dispensing the PTM is complete, discard the pipette tip and any remaining PTM.

C. Preparation of the Individual Donor Lysate

1. Mix all whole blood samples preferably on a tube mixer for at least 5 minutes or by inversions for at least 15 times. Ensure that the whole blood is mixed gently and thoroughly and is homogenous.

2. Remove the cap from the whole blood specimen and transfer 900 µL of whole blood into the pre-labeled lysate tube containing PTM. Following the dispense of the blood sample into the lysate tube, homogenize the whole blood and PTM by gently aspirating and dispensing the solution at least 5 times. Avoid creating bubbles or aerosols.

3. Dispose of the pipette tip into the container of 0.5–0.7% sodium hypochlorite.

4. Recap the whole blood specimen.

5. Repeat steps C. 2 to C. 4 for each separate lysate being prepared.

6. The lysate tube(s) are now ready to be placed into Panther Sample Racks and loaded on the Procleix Panther System for testing.

PROCEDURAL NOTES

Note: Refer to the *Procleix Panther System Operator's Manual* for operating instructions.

A. The operator must ensure that the Procleix Babesia Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the master lot barcode sheet enclosed with each shipment of Procleix Babesia Assay Calibrators.

B. Replace bottles in the Universal Fluids Drawer when notified by the system. Refer to the *Procleix Panther System Operator's Manual*.

Note: Auto Detect Reagents and Assay Fluids may be used with any master lot of Procleix Assay Reagents that are run on the Procleix Panther System.

C. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15°C. Wash Solution may be warmed to facilitate dissolution of precipitate. **Do not use the RPI 250 or RES to warm the Wash Solution.** Temperature should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

D. To reduce the risk of invalid results, carefully read the entire package insert for the Procleix Babesia Assay prior to performing an assay run. This package insert must be used with the *Procleix Panther System Operator's Manual*, *Procleix Reagent Preparation Incubator 250 Operator's Manual* or *Procleix Reagent Equilibration System Operator's Manual,* and any applicable technical bulletins.

E. RUN SIZE

For the Procleix Babesia Assay, each worklist may contain up to 250 tests, including Procleix Babesia Assay Calibrators.

F. EQUIPMENT PREPARATION

See the *Procleix Panther System Operator's Manual*.

G. RUN CONFIGURATION

1. Each run must have a set of Procleix Babesia Assay Calibrators.
2. For the Procleix Babesia Assay, a set of calibrators consists of one vial each of Negative Calibrator and Positive Calibrator. The Negative and Positive Calibrators are run in triplicate.

H. WORK FLOW

1. Prepare reagent in clean area.
2. The sample loading area must be amplicon-free.

I. DECONTAMINATION

1. The extremely sensitive detection of analytes by this test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5 to 0.7% sodium hypochlorite in water (diluted bleach). Allow bleach to contact surfaces for at least 15 minutes, then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. Follow instructions provided in the *Procleix Panther System Operator's Manual* for instrument decontamination and maintenance procedures.

ASSAY PROCEDURE

Procleix Babesia Assay Calibrators are to be used with the corresponding master lot of the Procleix Babesia Assay. The operator must check to ensure that the Procleix Babesia Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the Procleix Babesia Assay master lot sheet in use.

For equipment preparation, rack setup, and assay procedure information, see instructions in the *Procleix Panther System Operator's Manual*.

QUALITY CONTROL PROCEDURES

Note: All Quality Control procedures described below are performed by the Procleix Babesia Assay software.

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX BABESIA ASSAY

A. Run validity:

A run (also identified as a worklist) is valid if the minimum number of calibrators meet their acceptance criteria and are valid (see section II below).

1. In a Procleix Babesia Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.
2. Calibrator acceptance criteria are automatically verified by the Procleix Panther System Software. If less than the minimum number of calibrator replicates is valid, the Procleix Panther System Software will automatically invalidate the run.
3. In a valid run, cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower).
4. If a run is invalid, sample results are reported as invalid and all specimens must be retested.

B. Sample validity:

1. In a valid run, a sample result is valid if the IC signal is equal to or above the IC cutoff, with the following exceptions:
   a. Specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control (IC) signal is below the cutoff.

b. Specimens with an IC signal above 750,000 RLU are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates Positive Calibrators with an IC signal above 750,000 RLU.

2. A sample may also be invalidated due to instrument and results processing errors. Refer to the *Procleix Panther System Operator's Manual* for details.
3. All individual specimen results that are invalid in a valid run must be retested.
4. When an invalid result is obtained with cadaveric lysate in a valid run, cadaveric lysate may be diluted 1:4 in PTM (i.e., 0.3 mL lysate plus 1.2 mL PTM). Diluted specimens should be inverted several times to mix and then may be used in the standard assay procedure.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the Procleix Babesia Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 400,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values.

The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Positive Calibrator Acceptance Criteria

The Positive Calibrator is run in triplicate in the Procleix Babesia Assay. Individual Positive Calibrator (PC) analyte values must be less than or equal to 3,000,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 750,000 RLU. If one of the Positive Calibrator replicate values is outside these limits, the Positive Calibrator mean (PC_x) will be recalculated based upon the two acceptable Positive Calibrator replicate values. The run is invalid and must be repeated if two or more of the three Positive Calibrator analyte values are outside of these limits.

Summary of Acceptance Criteria for Procleix Babesia Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 400,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 3,000,000 RLU
Internal Control	≤ 750,000 RLU

Summary of Cutoff Calculations for Procleix Babesia Assay

Analyte Cutoff =	NC Analyte Mean RLU + [0.08 X (PC Analyte Mean RLU)]
Internal Control Cutoff =	0.5 X (Negative Calibrator IC Mean RLU)

INTERPRETATION OF RESULTS

Note: All calculations described below are performed by the Procleix Panther System Software.

Two cutoffs are determined for each assay: one for the Analyte Signal (glower signal) termed the Analyte Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 750,000 RLU. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO $\geq$ 1.00) and the IC Signal is less than or equal to 750,000 RLU. Reactive results will be designated by the software. A specimen is invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is less than the Internal Control Cutoff. Any specimen with Internal Control values greater than 750,000 RLU is considered Invalid and the reactive status cannot be assessed.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
Nonreactive	Analyte S/CO < 1.00 and IC $\geq$ IC Cutoff and IC $\leq$ 750,000 RLU
Reactive	Analyte S/CO $\geq$ 1.00 and IC $\leq$ 750,000 RLU*
Invalid	IC $>$ 750,000 RLU or Analyte S/CO < 1.00 and IC $<$ Cutoff

*For specimens with IC signal greater than 750,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

A. Any specimen with an interpretation of Invalid or Error in the Procleix Babesia Assay must be retested in singlet.

B. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate lysate from the index donation may be used as long as the storage criteria in the package insert are met.

C. Lysates with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the Procleix Babesia Assay are considered Nonreactive for *Babesia*.

No further testing of a *Babesia* Nonreactive specimen is required.

D. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC Signal less than or equal to 750,000 RLU are considered Reactive.

1. If an individual donor lysate tests Reactive with the Procleix Babesia Assay, then the individual donor lysate is considered Reactive for *Babesia*.
2. If a pooled donor lysate tests Reactive with the Procleix Babesia Assay, then the pooled donor lysates must be deconvoluted by testing the individual donor lysates that were combined in this pool.
3. Further clarification of the Reactive lysates for informational purposes may be obtained by testing an alternate lysate from the index donation with the Procleix Babesia Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
4. Any Reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

LIMITATIONS OF THE PROCEDURE

A. This assay has been developed and intended for use with the Procleix Panther System only.

B. Test results may be affected by improper specimen collection, storage, or specimen processing.

C. Cross-contamination of samples can cause false positive results.

D. Assays must be performed, and results interpreted, according to the procedures provided.

E. Deviations from these procedures, adverse shipping and/or storage conditions, or use of outdated reagents may produce unreliable results.

F. Failure to achieve expected results is an indication of an invalid run. Possible sources of error include test kit deterioration, operator error, faulty performance of equipment, specimen deterioration, or contamination of reagents.

G. Certain substances may interfere with the performance of the assay. See the Specificity and Sensitivity of the Procleix Babesia Assay in the Presence of Donor and Donation Factors section.

H. Though rare, mutations within the highly conserved regions of the genome covered by the primers and/or probes in the Procleix Babesia Assay may result in failure to detect the parasite.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS IN LIVING DONOR BLOOD SPECIMENS

Clinical Performance

Clinical Reproducibility of the Procleix Babesia Assay

Reproducibility was evaluated on the Procleix Panther System at 3 U.S. sites (1 in-house and 2 external). Two operators performed testing using 1 Procleix Panther System at each site. Each operator performed 3 runs per day on each of 6 days, using 3 reagent lots equally over the course of testing. Each run had 2 replicates of each panel member.

The negative panel members were made from *Babesia*-negative lysed human whole blood. The positive panel members were created by spiking the whole blood with *B. microti* infected hamster whole blood diluted in human whole blood. Very low positive, low positive, moderate positive, and high positive concentrations were prepared for testing.

Agreement values were 100% in the negative, low, moderate, and high positive panel members and 67.91% in the very low positive panel member.

Table 1 shows the percent agreement with expected results and reproducibility and precision of assay results for each panel member between sites/ instruments, between operators, between lots, between days, within run, and overall.

Table 1. Reproducibility of the Procleix Babesia Assay on the Procleix Panther System

Panel*	Average S/CO	% Agrmt	Between Sites/ Instruments		Between Operators		Between Lots		Between Days		Within Run		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative	<0.01	100	0.01	170.05	<0.01	48.03	<0.01	21.54	0	0	0.01	162.38	0.02	240.95
Very Low Positive**	8.08	67.91	0	0	0	0	0	0	0.17	2.09	6.02	74.42	6.02	74.45
Low Positive	13.10	100	0.22	1.66	0.09	0.70	0.25	1.92	0.45	3.41	0.77	5.88	0.96	7.29
Moderate Positive	13.16	100	0	0	0.13	1.02	0.17	1.26	0.42	3.17	0.69	5.27	0.84	6.36
High Positive	13.24	100	0	0	0	0	0.18	1.37	0.54	4.07	0.69	5.20	0.89	6.75

S/CO = Analyte signal to cutoff ratio, Agrmt = Agreement, SD = Standard Deviation, CV = Coefficient of variation

Note: Variability from some factors may be numerically negative. This can occur if the variability due to those factors is very small. In these cases, SD and %CV are shown as 0.

*n = 214 for the low positive and high positive panel members, 215 for the negative and very low positive panel members, and 216 for the moderate positive panel member.

Estimated target concentration in parasites/mL (p/mL) for each panel member: Negative = 0 p/mL, Very Low Positive = 2.60 p/mL, Low Positive = 15.61 p/mL, Moderate Positive = 52.05 p/mL, High Positive = 260.23 p/mL

**The very low positive panel member was expected to have <100% reactive results, as it had a concentration targeted below the assay's 95% limit of detection for the hamster blood stock used to prepare panel members.

Clinical Specificity of the Procleix Babesia Assay

Specificity in Normal Blood Donors

A prospective, multicenter clinical study was conducted to estimate specificity of the Procleix Babesia Assay on the Procleix Panther System. Whole blood samples from voluntary whole blood donors were lysed and tested in 16-sample pools and/or individually. Three US blood testing laboratories performed testing. A minimum of 3 reagent kit lots were used over the course of the study at each testing laboratory.

Pools were created by combining aliquots from 16 individual lysates. For determination of true positive status, samples from individual donations with an initial reactive result were tested for *B. microti* DNA with a polymerase chain reaction (PCR) assay and for immunoglobulin G (IgG) antibody with an immunofluorescence assay (IFA) using a cutoff of 1.64; samples with undetected or negative *B. microti* comparator assay results were tested with a PCR assay that also detects *B. duncani, B. divergens,* and *B. venatorum*. Donors of lysates with initially reactive results were asked to enroll in follow-up testing. Donor status was based on the Procleix Babesia Assay result for Procleix Babesia Assay nonreactive donations, and testing of the index donation with the comparator assays, when samples had reactive Procleix Babesia Assay results.

Of the 509 Procleix Babesia Assay runs, 502 runs (98.62%, 502/509) were valid and 7 runs (1.38%, 7/509) were invalid due to operator error (n=2) or hardware error (n=5). Of the 11,068 individual donations, 1 individual donation (<0.01%, 1/11,068) had an initial invalid result due to hardware error, was not retested, and was excluded from the analyses. Of the 11,058 sixteen-sample pools, 19 pools (0.17%, 19/11,058) had initial invalid results due to operator error (13 results in 1 run) or hardware errors (6 results in 5 runs), were not retested, and were excluded from the analyses. One (1) additional

Procleix Babesia assay nonreactive 16-sample pool had an unknown final interpretation because one of the individual donations used to prepare the pool had an invalid result and the remaining 15 donations had nonreactive results; this pool was excluded from analyses.

A total of 11,067 individual donations and 11,038 sixteen-sample pools consisting of 176,608 individual donations had final outcomes for the specificity analyses. Procleix Babesia Assay specificity was 100% (11,060/11,060; 95% CI: 99.97% to 100%) in individual donations and 100% (10,977/10,977; 95% CI: 99.97% to 100%) in 16-sample pools.

Table 2 shows the specificity of the Procleix Babesia Assay on the Procleix Panther System in individual donations.

Table 2. Clinical Specificity of the Procleix Babesia Assay on the Procleix Panther System in Individual Donations

Sample Type	n	True Negative	True Positive	False Positive	% Specificity	95% CI
Individual donations	11,067	11,060	7	0	100	99.97-100
16-sample pools	11,038	10,977	60*	0	100	99.97-100

n = number of specimens
CI = Clopper-Pearson confidence interval
*Includes 6 true positive donations identified in individual donation testing; 1 individual donation with an initial reactive Procleix Babesia assay result confirmed by IFA was not detected in a 16-sample pool.

Clinical Sensitivity of the Procleix Babesia Assay

B. microti, B. duncani, B. divergens, and *B. venatorum* known-positive whole blood specimens and cultured erythrocytes were used to prepare lysates neat (i.e., undiluted) and in a 1:16 dilution. Known-positive status was verified by testing 1:16 diluted samples with a NAT. The neat and diluted samples were tested with the Procleix Babesia Assay at three laboratories (1 in-house and 2 external). Three clinical reagent kit lots were used at each site. Results were compared to the known-positive status and clinical sensitivity was calculated (Table 3).

Of the 9 runs performed, all (100%, 9/9) were valid. All neat and diluted samples had valid results.

Procleix Babesia assay sensitivity was 100% (131/131; 95% CI: 97.22% to 100%) in both neat (undiluted) and diluted (1:16) known-positive samples.

Table 3. Clinical Sensitivity of the Procleix Babesia Assay in Known-Positive Samples

Sample Type	n	True Positive	False Negative	% Sensitivity	95% CI
Neat	131	131	0	100	97.22-100
Diluted	131	131	0	100	97.22-100

n = number of specimens
CI = Clopper-Pearson confidence interval

Analytical Performance

Analytical Sensitivity

Assay sensitivity was evaluated with serially diluted *in vitro* transcript based on the sequence corresponding to the 18s ribosomal RNA of *B. microti, B. divergens, B. duncani,* and *B. venatorum* (GenBank Accession numbers AY693840, AY789076, AY027815, and AY046575 respectively). Assay sensitivity for the detection of *B. microti, B. divergens,* and *B. duncani* was also evaluated with serially diluted infected erythrocytes in human whole blood, prior to lysis in PTM using 0.9 mL of whole blood in 2.7 mL of PTM. Each lysate was tested in singlet. The panels were tested with the Procleix Babesia Assay on the Procleix Panther System.

The average analyte S/CO ratio and percent coefficient of variation (%CV) for samples containing viral RNA or *in vitro* transcript were calculated from concordant results only (S/CO>1.0). The 95% confidence intervals of the reactivity rates were based on the Score method[19]. Estimations of 50% and 95% detection rates were determined by Probit Analysis.

The detection rates for *Babesia in vitro* transcripts are shown in Table 4, 5, 6, and 7. The detection rates for *Babesia* parasites are shown in Table 8, 9, and 10.

Table 4. Detection of *B. microti in vitro* Transcript

B. microti in vitro Transcript, copies/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
100	72/72	100 (94.93–100)	12.14	5.67
30	72/72	100 (94.93–100)	11.17	12.52
10	69/72	95.83 (88.45–98.57)	8.65	33.39
3	48/72	66.67 (55.18–76.47)	7.00	47.19
1	19/72	26.39 (17.59–37.58)	6.35	53.65
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 5. Detection of *B. divergens in vitro* Transcript

B. divergens in vitro Transcript, copies/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
100	72/72	100 (94.93–100)	11.73	8.60
30	72/72	100 (94.93–100)	10.21	20.14
10	66/72	91.67 (82.99–96.13)	7.75	39.73
3	27/72	37.50 (27.22–49.05)	6.03	55.06
1	8/72	11.11 (5.74–20.42)	6.31	43.88
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 6. Detection of *B. duncani in vitro* Transcript

B. duncani in vitro Transcript, copies/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
100	72/72	100 (94.93–100)	11.54	10.37
30	72/72	100 (94.93–100)	10.48	14.13
10	72/72	100 (94.93–100)	8.53	28.02
3	40/72	55.56 (44.09–66.46)	6.00	46.55
1	13/72	18.06 (10.87–28.48)	6.64	40.44
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 7. Detection of *B. venatorum in vitro* Transcript

B. venatorum in vitro Transcript, copies/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
100	72/72	100 (94.93–100)	11.46	5.67
30	72/72	100 (94.93–100)	10.01	12.52
10	65/72	90.28 (81.27–95.21)	8.05	33.39
3	37/72	51.39 (40.07–62.57)	6.61	47.19
1	14/72	19.44 (11.95–30.03)	6.29	53.65
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 8. Detection of *B. microti* Parasite

B. microti parasite, parasite/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
6	72/72	100 (94.93–100)	13.48	4.76
4	72/72	100 (94.93–100)	13.34	6.01
2	59/75	81.94 (71.52–89.13)	13.22	4.69
1	46/72	63.89 (52.35–74.02)	12.61	17.25
0.5	36/72	50.00 (38.75–61.25)	13.17	9.76
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 9. Detection of *B. divergens* Parasite

B. divergens parasite, parasite/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
6	72/72	100 (94.93–100)	12.97	8.72
4	72/72	100 (94.93–100)	12.17	18.55
2	69/72	95.83 (88.45–98.57)	10.87	31.41
1	59/72	81.94 (71.52–89.13)	10.65	33.84
0.5	36/72	50.00 (38.57–61.25)	9.06	47.07
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Table 10. Detection of *B. duncani* Parasite

B. duncani parasite, parasite/mL	Number of Reactive/ Tested	% Reactive (95% CI)	Average S/CO	% CV
6	72/72	100 (94.93–100)	10.73	16.72
4	71/72	98.61 (92.54–99.75)	10.13	23.61
2	58/72	80.56 (69.97–88.05)	8.32	35.66
1	48/72	66.67 (55.18–76.47)	7.12	44.06
0.5	27/72	37.50 (27.22–49.05)	7.07	46.83
0	0/72	0 (0–5.07)	0.00	N/A

CI = Score Confidence Interval, CV = Coefficient of Variation, S/CO = Signal to Cutoff ratio in concordant replicates only, N/A = Not Applicable

Probit Analysis

The 50% and 95% detection probabilities for *Babesia in vitro* transcript and *Babesia* parasite were determined by Probit Analysis, using the Gompertz model.[20] For the *Babesia in vitro* transcript, the 50% LOD estimates ranged from 2.06 to 3.90 copies/mL and the 95% LOD estimates ranged from 6.73 to 12.44 copies/mL (Table 11). For the *Babesia* parasite, the 50% LOD estimates ranged from 0.48 to 0.72 parasites/mL and the 95% LOD estimates ranged from 1.77 to 3.10 parasites/mL (Table 12).

Table 11. Detection Probabilities of *Babesia in vitro* Transcript

Babesia species	Detection Probabilities, copies/mL	
	50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
B. microti	2.06 (1.55–2.56)	8.91 (6.88–12.88)
B. divergens	3.90 (3.21–4.62)	11.58 (9.38–15.44)
B. duncani	2.54 (2.11–3.01)	6.73 (5.32–9.69)
B. venatorum	2.99 (2.33–3.67)	12.44 (9.62–17.87)

Table 12. Detection Probabilities of *Babesia* Parasite

Babesia species*	Detection Probabilities, parasites/mL	
	50% (95% Fiducial Limits)	95% (95% Fiducial Limits)
B. microti	0.61 (0.43–0.76)	2.98 (2.40–4.09)
*B. divergens***	0.48 (0.34–0.60)	1.77 (1.45–2.41)
*B. duncani***	0.72 (0.54–0.88)	3.10 (2.53–4.11)

* Infected red blood cells were counted by microscopy or by Fluorescence-Activating Cells Sorting (FACS)

** *B. divergens* and *B. duncani* were washed in Phosphate-Buffered Saline (PBS) prior dilution in human whole blood to remove free parasitic ribosomal RNA.

Specificity and Sensitivity of the Procleix Babesia Assay in the Presence of Donor and Donation Factors

When tested with the Procleix Babesia Assay, no cross-reactivity or interference was observed for naturally occurring hemolyzed or lipemic whole blood specimens or whole blood specimens with the following substances added: albumin (60,000 mg/L), hemoglobin (5,000 mg/L), bilirubin (200 mg/L), gammaglobulin (60,000 mg/L), or lipids (30,000 mg/L). Specificity and sensitivity were 100%.

No cross-reactivity or interference was observed in whole blood specimens from patients with autoimmune or other diseases not caused by *Babesia* infection. Multiple whole blood specimens from each group of patients with the following autoimmune or other conditions were evaluated: alcoholic

cirrhosis, elevated alanine amino transferase, rheumatoid factor, antinuclear antibody, systemic lupus emphysematous, multiple sclerosis, and multiple myeloma. Specificity and sensitivity were 100% and 98.81%, respectively. For sensitivity, no statistical difference using Fisher's exact test was observed between autoimmune or other diseases and control specimens ($p \leq 0.46$).

No cross-reactivity or interference was observed in whole blood specimens contaminated with bacteria or fungi. Whole blood specimens spiked with the following microorganisms were evaluated: *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Corynebacterium diphtheriae, Propionibacterium acnes, Candida albicans, Pneumocystis carinii, Borelia burgdorferi, Trypanosoma cruzi, Trypanosoma rangeli,* and *Leptospira interrogans.* Specificity and sensitivity were 100%.

No cross-reactivity or interference was observed in whole blood specimens from subjects infected with other blood-borne pathogens, or those that had received Flu and HBV vaccines. Multiple whole blood specimens from each group of the following infections were evaluated: dengue virus types 1–4, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus 1 and 2 (HIV 1/2), parvovirus B-19, West Nile virus (WNV), hepatitis E virus (HEV), chikungunya (CHIKV), cytomegalovirus (CMV), human T-lymphotropic virus 1 and 2 (HTLV-1/2), influenza virus H1N1, *plasmodium falciparum,* and influenza and HBV vaccinated individuals. Specificity and sensitivity were 100%.

No cross-reactivity or interference was observed when equal volume pools of 16 donor and donation factor specimens were tested in the Procleix Babesia Assay. Specificity and sensitivity were 100%. A higher incidence of initial invalid results of 9.80% was observed in pools of 16 donations, each containing combinations of the potentially problematic samples described above.

Specificity and Sensitivity of the Procleix Babesia Assay in the Presence of Exogenous Substances

No cross-reactivity or interference was observed in whole blood containing the following substances: Acetaminophen (1324 µmol/L), Acetylsalicylic Acid (3620 µmol/L), Ascorbic Acid (342 µmol/L), Atorvastatin (600 µg Eg/L), Ibuprofen (2425 µmol/L), Loratadine (0.78 µmol/L), Naproxen (2170 µmol/L), and Phenylephrine HCl (491 µmol/L).

PERFORMANCE CHARACTERISTICS IN CADAVERIC DONOR BLOOD SPECIMENS

Reproducibility of the Procleix Babesia Assay in Cadaveric Blood Specimens on the Procleix Panther System

The inter-assay reproducibility of the Procleix Babesia Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 20 cadaveric and 20 normal blood donor (control) whole blood specimens spiked with *Babesia* parasite were tested on the Procleix Panther System. Each specimen was tested individually, in 6 separate runs, on 6 separate days using 3 reagent lots with a minimum of 2 Procleix Panther Systems. The reactive rates, S/COs, and %CVs are shown in Table 13.

The %CVs for cadaveric and control whole blood specimens were 11.79% and 4.80%, respectively. The percent reactive rates for the cadaveric and control whole blood specimens were 99.73% and 100%, respectively. For the percent reactive rates, no statistical difference using Fisher's exact test was observed between cadaveric and control specimens ($p \leq 1.00$). The intra-run variability component of the reproducibility analysis showed the largest source of variability for both cadaveric and control specimen mean analyte S/CO. A higher incidence of initial invalid results of 5.12% was observed in cadaveric specimens. All specimens were valid upon retest.

Table 13. Summary of Reproducibility Testing of the Procleix Babesia Assay in Cadaveric and Control Specimens

Sample	# Donors	# Valid Replicates	% Reactivity (95% CI)	Mean Analyte S/CO	%CV
Cadaveric	20	370*	99.73 (98.50–99.99)	12.99	11.79
Control	20	360	100 (98.98–100)	12.81	4.80

CI = Clopper-Pearson Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
CV = Coefficient of Variation in concordant replicates only
* Additional testing was performed inadvertently, on Day 5, resulting in 10 valid tests included in the analysis.

Specificity of the Procleix Babesia Assay in Cadaveric Blood Specimens

Cadaveric blood specimens were tested to determine the specificity of the Procleix Babesia Assay. Fifty cadaveric specimens and 50 normal blood donor (control) whole blood specimens were tested. The specificity for the cadaveric and control specimens was 100% (95% CI: 92.89%–100%; Table 14). A higher incidence of initial invalid results of 18.00% was observed in cadaveric specimens. All specimens were valid upon retest.

Table 14. Specificity of the Procleix Babesia Assay in Cadaveric Blood Specimens

	Control	Cadaveric
Mean IC S/CO	2.06	2.08
Mean Analyte S/CO	0.00	0.00
% Specificity (95% CI)	100 (92.89–100)	100 (92.89–100)
n	50	50

n = Number of tests
IC = Internal Control
CI = Clopper-Pearson Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only

Sensitivity of the Procleix Babesia Assay in Cadaveric Blood Specimens Spiked with *Babesia*

Cadaveric blood specimens were tested to determine the sensitivity of the Procleix Babesia Assay. Fifty cadaveric specimens and 50 normal blood donor (control) whole blood specimens were tested. The sensitivity for the cadaveric and control specimens was 98.00% (95% CI: 89.35%–99.95%) and 100% (95% CI: 92.89%–100%), respectively (Table 15). For the sensitivity, no statistical difference using Fisher's exact test was observed between cadaveric and control specimens ($p \leq 1.00$). A higher incidence of initial invalid results of 6.00% was observed in cadaveric specimens. All specimens were valid upon retest.

Table 15. Sensitivity of the Procleix Babesia Assay in Cadaveric Blood Specimens

	Control	Cadaveric
Mean IC S/CO	N/A	N/A
Mean Analyte S/CO	13.16	13.24
% Reactivity (95% CI)	100 (92.89–100)	98.00 (89.35–99.95)
n	50	50*

N/A = Not applicable for reactive results
n = Number of tests
IC = Internal Control
CI = Clopper-Pearson Confidence Interval
S/CO = Signal to Cutoff ratio in concordant replicates only
* False negative sample yielded two reactive results in duplicate retesting (not included in the analysis)

Stability of *Babesia* in Cadaveric Specimens

Stability of *Babesia* was evaluated in 10 unique cadaveric donors by spiking whole blood with *B. microti* at a level corresponding to approximately 3-fold the 95% limit of detection of the Procleix Babesia Assay. After spiking, whole blood specimens were subjected to a series of incubations prior lysis in PTM to evaluate stability in whole blood for up to 8 days. On each day of testing, the whole blood specimens were brought to room temperature, lysed in PTM, and tested on the same day. Individual lysates were prepared from each donor and tested on Day 0. The whole blood was incubated at 25°C for 24 hours, followed by incubation at 2°–8°C for up to 7 days. Individual lysates from each donor were prepared and tested on Day 1, Day 5, and Day 8. Each lysate was tested in duplicate at each time point.

Stability was also evaluated in individual lysates prepared from the 10 unique cadaveric donors. Multiple individual lysates prepared from whole blood from each donor on Day 5 and Day 8 were subjected to a series of incubations to evaluate stability of the lysate for up to 7 days. The lysates were incubated for 24 hours at 30°C, followed by 2 days at 25°C, then 4 days at 2°–8°C. A separate lysate tube for each donor was tested at Day 1, Day 4, and Day 7. On Day 7, the lysates were frozen at ≤-20°C. The frozen lysates for each donor were tested after 3 freeze thaw cycles. Each lysate was tested in duplicate at each time point.

The reactivity rates for cadaveric whole blood specimens were 95.45% on Day 0 and 100% on Day 1, Day 5, and Day 8. The reactivity rates for cadaveric lysates were 100% on Day 0, Day 4, Day 7, and Day 7 following 3 freeze thaw cycles. Lysates evaluated in this study were prepared from both 5 day old and 8 day old whole blood. The reactivity results were compared to Day 0 results using Fisher's Exact test and yielded p-values of 1.00 for specimens' evaluation cadaveric whole blood and lysate stability demonstrating no statistically significant differences. A higher incidence of initial invalid results of 2.50% was observed in cadaveric specimens. All specimens were valid upon retest. The results from testing the stability of *Babesia* in cadaveric whole blood and lysate specimens are summarized in Tables 16 and 17, respectively.

Table 16. Stability of *B. microti* in Cadaveric Whole Blood Specimens

Timepoint	Day 0	Day 1	Day 5	Day 7
# Valid	22	20	20	20
# Reactive	21	20	20	20
% Reactivity	95.45 (77.16-99.89)	100 (83.16-100)	100 (83.16-100)	100 (83.16-100)

CI = Clopper-Pearson Confidence Interval

Table 17. Stability of *B. microti* in Cadaveric Individual Lysate Specimens

Whole Blood	Timepoint	Day 0	Day 4	Day 7	Day 7 + 3 F/T
5 Day Old	# Valid	20	20	20	20
	# Reactive	20	20	20	20
	% Reactivity (95% CI)	100 (83.16-100)	100 (83.16-100)	100 (83.16-100)	100 (83.16-100)
8 Day Old	# Valid	20	20	20	20
	# Reactive	20	20	20	20
	% Reactivity (95% CI)	100 (83.16-100)	100 (83.16-100)	100 (83.16-100)	100 (83.16-100)

CI = Clopper-Pearson Confidence Interval
F/T = Freeze/Thaw

Stability of *Babesia* in Frozen Cadaveric Lysate Specimens

Stability of *Babesia* was evaluated in 10 unique cadaveric donors by spiking whole blood with *B. microti* at a level corresponding to approximately 3-fold the 95% limit of detection of the Procleix Babesia Assay. After spiking, multiple individual lysates were prepared from each donor and frozen at ≤-20°C for up to 12 months. A separate lysate tube for each donor was tested on Day 0, and at 6, 9, and 12 months. Each lysate was tested in triplicate at each time point.

The reactivity rates for frozen cadaveric lysate specimens were 100% at Day 0, 6 months, and 9 months, and 93.75% at 12 months. These reactivity results were compared to Day 0 results using Fisher's Exact test and yielded p-values of greater than or equal to 0.49 demonstrating no statistically significant differences. A higher incidence of initial invalid results of 13.33% was observed in cadaveric specimens. All specimens were valid upon retest. The results from testing the stability of *Babesia* in frozen cadaveric lysate specimens are summarized in Table 18.

Table 18. Stability of *B. microti* in Frozen Cadaveric Individual Lysate Specimens

Timepoint	Day 0	6 months	9 months	12 months
# Valid	30	30	30	32
# Reactive	30	30	30	30
% Reactivity	100 (88.43-100)	100 (88.43-100)	100 (88.43-100)	93.75 (79.19-99.23)

CI = Clopper-Pearson Confidence Interval

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Package Insert Master Document Revision History

Version number	Date	Changes
1.0	23AUG2024	Original version, sourced from GDSS-LD-IFU-000196 v. 6.0
2.0	23JUNE2025	Storage and Handling Information • Added point H " To reduce the possibility of reagent cross-contamination, it is recommended to use new reagent caps when unloading reagent bottles from the Panther instrument and storing them." • Added point K "For instructions on preparation of reagents, see instructions under REAGENT PREPARATION and the Procleix RPI 250 Operator's Manual or the Procleix Reagent Equilibration System Operator's Manual." • Updated point L (table) to reflect the revised storage duration for Parasite Transport Medium (PTM) room and storage temperature extending it from 30 days to 90 days per GDSS-RD-DVER-000432 Materials • Added purchasing information for Auto Detect 4k kit Specimen Collection, Storage, and Handling • Replaced figure captions with "the cumulative time at elevated temperatures cannot exceed these limits" Living Donor Whole Blood Specimens • In point F, replaced "It is recommended that pooled donor lysates are not frozen as reduced sensitivity may be observed" with "Pooled donor lysates should not be frozen." • Added point M "A validated pooling process may be used to perform pooling operations that combine aliquots from individual specimens into a single Master Pool Tube, which may be used for further testing. Refer to OTHER MATERIALS AVAILABLE FROM GRIFOLS FOR USE WITH PROCLEIX BABESIA ASSAY for Grifols validated pooling solutions." Cadaveric Donor Whole Blood Specimens • In point G, replaced "pooled donor lysates" with "lysates from cadaveric donor specimens." Limitations of the Procedure • Added point G " Certain substances may interfere with the performance of the assay. See the Specificity and Sensitivity of the Procleix Plasmodium Babesia Assay in the Presence of Donor and Donation Factors section" Added "Specimen Preparation" section