



Detection of *Babesia* RNA and DNA in whole blood samples from US blood donations

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Abstract

Background: Human babesiosis is a zoonotic infection caused by an intraerythrocytic parasite. The highest incidence of babesiosis is in the United States, although cases have been reported in other parts of the world. Due to concerns of transfusion-transmitted babesiosis, the US Food and Drug Administration (FDA) recommended year-round regional testing for *Babesia* by nucleic acid testing or use of an FDA-approved device for pathogen reduction. A new molecular test, cobas Babesia (Roche Molecular Systems, Inc.), was evaluated for the detection of the four species that cause human disease, *Babesia microti*, *Babesia duncani*, *Babesia divergens*, and *Babesia venatorum*.

Study design and methods: Analytical performance was evaluated followed by clinical studies on whole blood samples from US blood donations collected in a special tube containing a chaotropic reagent that lyses the red cells and preserves nucleic acid. Sensitivity and specificity of the test in individual samples (individual donation testing [IDT]) and in pools of six donations were determined.

Results: Based on analytical studies, the claimed limit of detection of cobas Babesia for *B. microti* is 6.1 infected red blood cells (iRBC)/mL (95% confidence interval [CI]: 5.0, 7.9); *B. duncani* was 50.2 iRBC/mL (95% CI: 44.2, 58.8);

Abbreviation: AltNAT1, alternative *Babesia* NAT test 1; AltNAT2, alternative *Babesia* NAT test 2; ARC, American Red Cross; *B. divergens*, *Babesia divergens*; *B. duncani*, *Babesia duncani*; *B. microti*, *Babesia microti*; *B. venatorum*, *Babesia venatorum*; CI, confidence interval; cp/mL, copies per milliliter; CPM, cobas PCR media; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; FDA, US Food and Drug Administration; IC, internal control; IDT, Individual donation testing; IFA, immunofluorescence assay; IND, Investigational New Drug Application; iRBC, infected red blood cells; iRBC/mL, infected red blood cells per milliliter; LoD, limit of detection; NAT, nucleic acid testing; neg-WB/CPM, *Babesia* negative whole blood in a ratio of 1:7 whole blood to CPM; NPA, negative percent agreement; PCR, polymerase chain reactivity; PPA, positive percent agreement; RMS, Roche Molecular Systems, Inc.; RNA, ribonucleic acid; rRNA, ribosomal RNA; RWBCT, Roche Whole Blood Collection Tube; TT, transfusion-transmitted; TTB, transfusion-transmitted babesiosis; US, United States.

List of cobas Babesia IND study group members is available in the Appendix.

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Roche Molecular Systems, Inc.

B. divergens was 26.1 (95% CI: 22.3, 31.8); and *B. venatorum* was 40.0 iRBC/mL (95% CI: 34.1, 48.7). The clinical specificity for IDT was 99.999% (95% CI: 99.996, 100) and 100% (95% CI: 99.987, 100) for pools of six donations.

Conclusion: cobas Babesia enables donor screening for *Babesia* species with high sensitivity and specificity.

KEYWORDS

Babesia, donor blood screening, nucleic acid testing (NAT)

1 | INTRODUCTION

Babesia is an intraerythrocytic parasite that is transmitted to humans via a tick-bite, most commonly of the *Ixodes* species¹⁻³ and may also be transmitted through the transfusion of blood and blood components, solid organ transplantation, or through vertical transmission from mother to child.⁴⁻⁹ Although most infected individuals are asymptomatic, symptoms of babesiosis range from mild to life-threatening. Severity may be increased in asplenic individuals, the elderly, the immunocompromised, and infants.^{1,2,6}

Babesia microti, *Babesia duncani*, *Babesia venatorum*, and *Babesia divergens* are known to cause human infection in different parts of the world.^{2,10-13} *B. microti*, *B. divergens*, and *B. venatorum* have been reported to cause human babesiosis in Europe¹⁴ and China,¹⁵ and *B. microti* or *B. microti*-like organisms have been reported in Singapore, Australia, Japan, Taiwan, and South Korea.^{14,16}

B. microti is the most common species in the United States where it is endemic to the Northeast and Upper Midwest of the United States; *B. duncani* has been reported along the Pacific coast and *B. divergens*-like organisms in Kentucky, Missouri, and Washington.^{2,3} In 2011, babesiosis became a nationally reportable disease in the United States,^{2,6} and the number of reported cases has since risen steadily.¹⁷ More than 200 cases of transfusion-transmitted babesiosis (TTB) in the United States have been reported due to *B. microti* primarily and *B. duncani*.^{6,8,18-20} To date, there have been no reports of TTB due to *B. divergens* and *B. venatorum*. In 2019, the US Food and Drug Administration (FDA) made recommendations to reduce the risk of TTB, including required screening of blood and blood components by nucleic acid testing (NAT) in the states at highest risk for *Babesia* or the use of an FDA-approved pathogen reduction device, which currently is only available for platelets and plasma.²¹

The performance of the cobas Babesia nucleic acid test for use on the cobas 6800/8800 Systems (Roche Molecular Systems, Inc.) for screening of whole blood samples is described, including clinical sensitivity and

specificity studies conducted on US blood donations by individual donation testing (IDT) and in pools of six individual donations.

2 | METHODS

2.1 | Assay and systems

The cobas Babesia test for use with the cobas 6800/8800 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 donors of blood and blood components and living organ and tissue donors.²² A volume of approximately 1.1 mL of whole blood sample is collected into the Roche Whole Blood Collection Tube (RWBCT) (Roche Molecular Systems, Inc.), a designated evacuated blood collection tube containing 7.7 mL of cobas PCR media (CPM), a preanalytic chaotropic reagent that will lyse red blood cells and preserve nucleic acid.²³ Following centrifugation, the RWBCT is the primary tube used for this test on the fully automated cobas 6800/8800 Systems. Whole blood samples may be tested either as individual samples or in pools composed of aliquots of individual samples using the cobas Synergy Software (Roche Molecular Systems, Inc.) and the Hamilton Microlab STAR (Hamilton Company) instrument. Synergy Software collates the results of pooling and testing, assigns the outcomes to individual samples according to a testing algorithm, and formats an output file that can be sent to a laboratory information system.

The cobas Babesia test master mix contains amplification primers and detection probes for *Babesia* and internal control (IC) nucleic acid. The specific *Babesia* probes, designed to detect ribosomal RNA and DNA of *B. microti*, *B. duncani*, *B. venatorum*, and *B. divergens*, and the IC detection probes are each labeled with one of two unique fluorescent dyes, which act as a reporter and a second dye that acts as a quencher. The reporter dye is measured at a defined wavelength, thus permitting simultaneous detection and

discrimination of the *Babesia* target and the IC. The assay is not intended to discriminate between the *Babesia* species.

2.2 | Nonclinical performance evaluation

2.2.1 | Analytical sensitivity

The analytical sensitivity of the cobas Babesia test was determined by testing the cultured strains of *B. microti*, *B. duncani*, *B. divergens*, and *B. venatorum*. Three independent dilution panels for each species were prepared to the appropriate concentration above, below, and at the expected limit of detection (LoD) of cobas Babesia by diluting positive samples in *Babesia* negative human ethylenediaminetetraacetic acid (EDTA) whole blood. *B. microti* (ATCC, Strain 30221) and *B. duncani* (ATCC, Strain PRA 302) infected red blood cells (iRBC) were obtained from fresh hamster blood. *B. divergens* (Oniris, Strain B128) and *B. venatorum* (Oniris, Strain C201) were obtained from fresh infected sheep blood. The concentration of iRBC was determined by manual counting using a hemocytometer and Giemsa staining to determine percent parasitemia. The panel members were inoculated into the RWBCTs. For each panel member, two different whole blood input volumes (1.1 and 0.4 mL) were added to the collection tubes. The 1.1 mL reflects

the expected direct draw volume with the RWBCTs. A reduced input volume of 0.4 mL was also tested for the maximum effect of high altitudes on blood volume collected by the tube.²⁴ Panels were tested over multiple reagent lots, days, operators, systems, and replicates per run. Approximately 126 replicates per concentration were tested. The resulting data were calculated by probit analysis (SAS Biometric Tools, SAS Institute, Inc.) to determine the 95% and 50% LoD in iRBC/mL for each species.

Analytical sensitivity was also determined by testing in vitro generated 18S RNA transcripts for each species. Panels were prepared from *Babesia* RNA transcripts serially diluted in buffer specimen diluent. Each panel of five concentration levels plus a blank was tested over multiple days, runs, and reagent lots for a total of 24 replicates per concentration level. Probit analysis on the combined data was used to estimate the 95% and 50% LoD in copies per milliliter (cp/mL) for each species.

2.2.2 | Secondary standards

Babesia secondary standards were prepared as dilutions of either *Babesia* infected hamster red blood cells (*B. microti*, *B. duncani*) or sheep red blood cells (*B. divergens*, *B. venatorum*) diluted in CPM using the stock titers provided by the vendors (ATCC or Oniris).

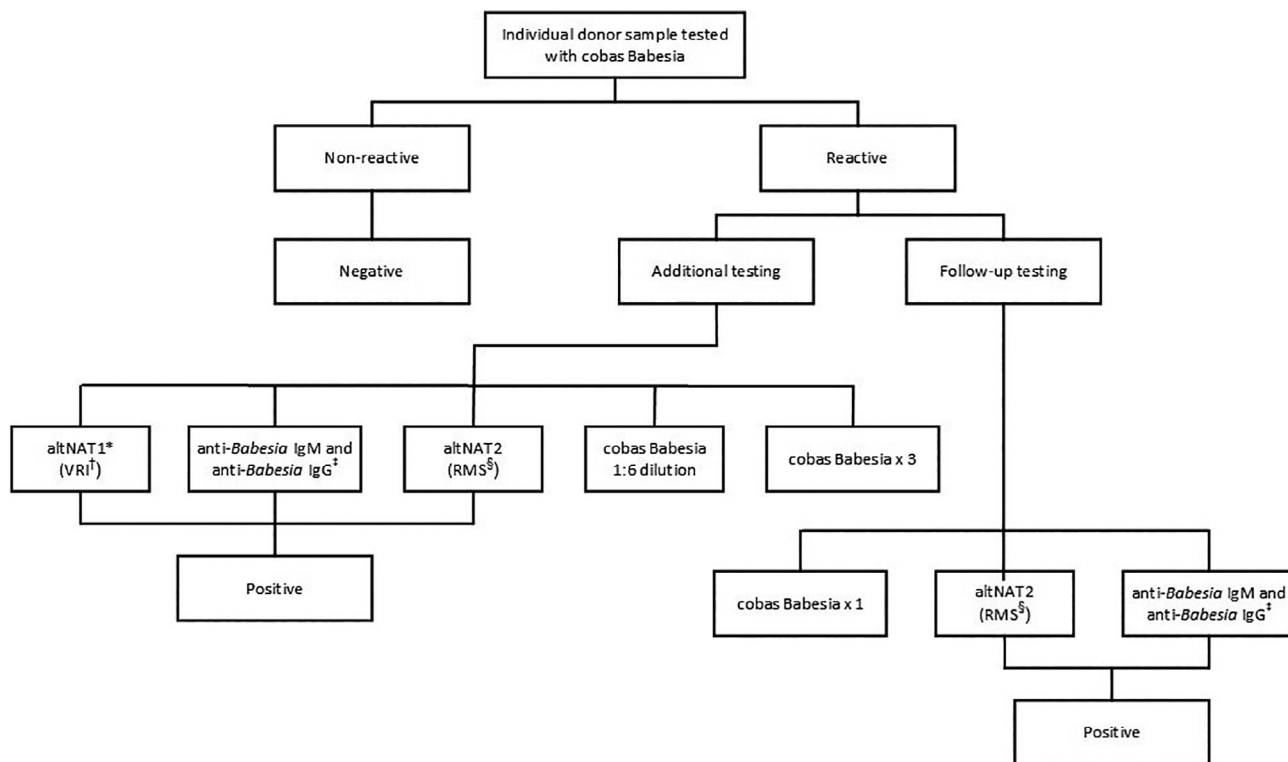


FIGURE 1 Index testing algorithm for individual donation samples. * altNAT = alternative NAT, † Vitalant Research Institute, ‡ Quest Diagnostics, § RMS = Roche Molecular Systems, Inc

2.2.3 | Analytical specificity

Analytical specificity was evaluated by testing cross reactivity with 15 microorganisms at 1.00E+05 to 1.00E+06 copies, genome equivalents, international units, or colony-forming units per mL including five parasites, five viral isolates, four bacterial strains, and one fungus. The microorganisms were added to *Babesia*-negative EDTA-whole blood specimens, which were prediluted with CPM in a ratio of 1:7 whole blood to CPM, to simulate the primary sample in the RWBCT (neg-WB/CPM). Each microorganism was tested in three replicates each with and without *B. microti* secondary standard added to a concentration of approximately 3× LoD of cobas Babesia. The reactive rate was calculated for each of the cross reactants with and without the addition of *B. microti*.

2.2.4 | Inclusivity

The inclusivity of cobas Babesia was determined by testing a total of 10 unique clinical specimens of *B. microti* neat

and diluted in neg-WB/CPM to approximately 4× LoD. Titers of clinical specimens were determined using the *B. microti* secondary standard. Due to the unavailability of clinical specimens, secondary standards were used for *B. duncani*, *B. divergens*, and *B. venatorum* diluted in neg-WB/CPM to approximately 4× LoD.

2.3 | Clinical performance evaluation

Seven testing sites participated in studies to determine the clinical sensitivity and specificity of cobas Babesia. These sites included the American Red Cross, Gaithersburg, MD; ImpactLife, Davenport, IA; Versiti Blood Center, Indianapolis, IN; Innovative Blood Resources, St. Paul, MN; Gulf Coast Regional Blood Center, Houston, TX; The Community Blood Center, Appleton, WI; and Central Pennsylvania Alliance Laboratory, York, PA. Testing was performed under an Investigational New Drug application approved by the FDA. Each test site obtained approval from their Institutional Review Board in accordance with the FDA.

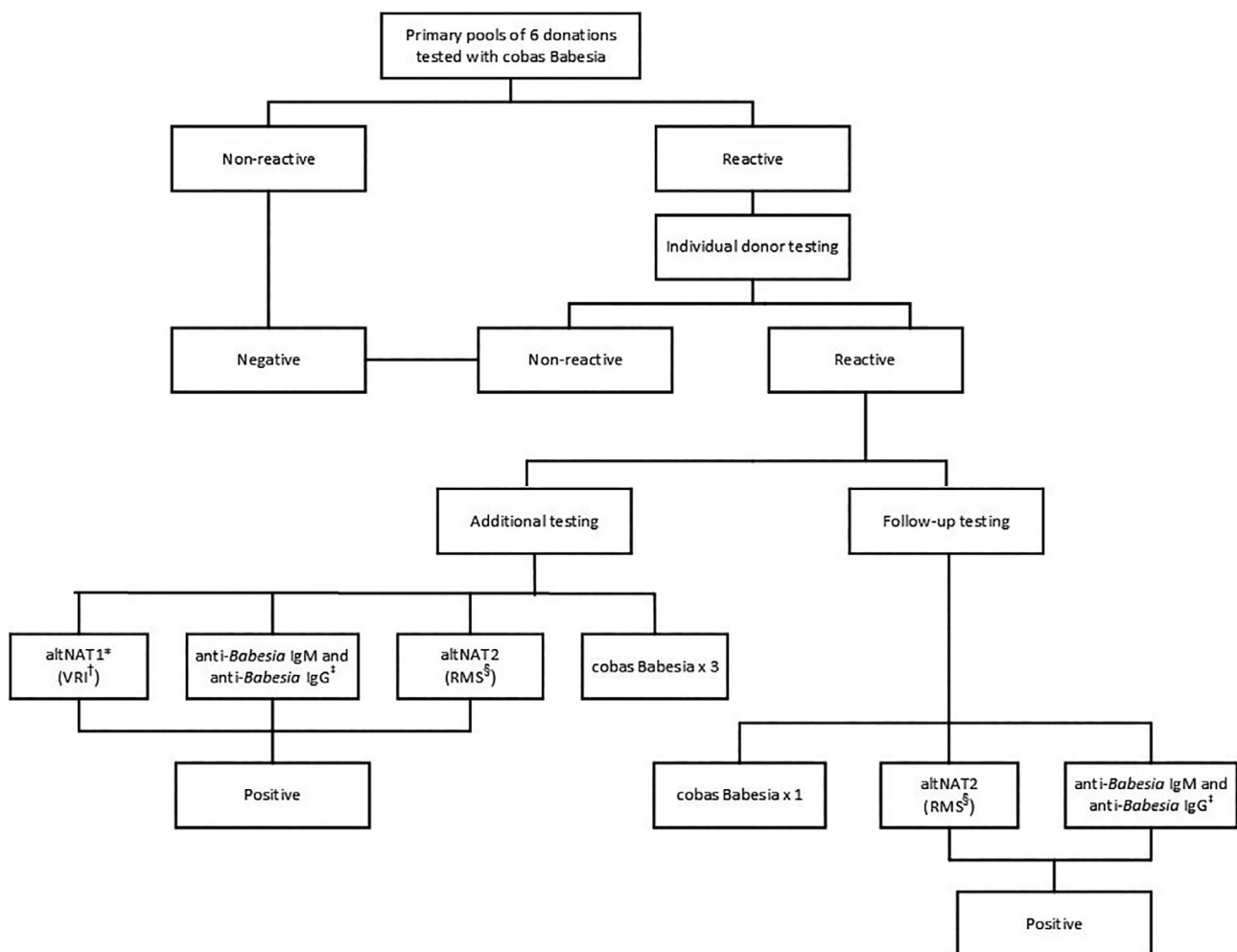


FIGURE 2 Index testing algorithm for pools of six donations. *altNAT = alternative NAT, †Vitalant Research Institute, ‡Quest Diagnostics. §RMS = Roche Molecular Systems, Inc

2.3.1 | Clinical sensitivity

A total of 203 specimens, composed of 131 clinical and 72 contrived specimens, known to be positive for *Babesia* by NAT were tested neat and in a 1:6 dilution in neg-WB/CPM to simulate a pool of 6 donations. Neat clinical samples were frozen remnant whole blood specimens positive for *B. microti* by a laboratory-developed NAT at Mayo Clinic, mixed with neg-WB/CPM at a 1:7 ratio.

Contrived samples were prepared by spiking neg-WB/CPM with secondary standards for each *Babesia* species at low, medium, and high concentrations of approximately 18 \times , 36 \times , and 72 \times LoD for neat samples.

A total of 1218 *Babesia*-positive samples were tested across three sites with cobas Babesia. The clinical sensitivity was calculated as the percentage of known positive samples detected, including the two-sided Clopper–Pearson exact 95% confidence interval (95% CI).²⁵

2.3.2 | Clinical specificity

Samples from donors who met standard donor eligibility criteria for donation were collected in the RWBCT.

Individual donation study

Whole blood samples were collected from October 2017 through September 2018 from US blood donors using the RWBCT and tested with cobas Babesia. The sites

were chosen to include different *Babesia* endemicity levels. States with high endemicity were those identified in the 2018 FDA Draft Guidance for Industry, “Recommendations for Reducing the Risk of Transfusion-Transmitted Babesiosis.”²⁶ States with reported cases of babesiosis that were not identified in the draft guidance were considered to be of low endemicity, and states that had no reports of babesiosis were considered to be nonendemic.

Additional testing was conducted on index donations reactive on cobas Babesia (Figure 1), including repeat testing in triplicate with cobas Babesia; simulated 1:6 pool by testing a dilution of the donor sample in neg-WB/CPM; two alternative *Babesia* NAT (AltNAT1, AltNAT2); and anti-*Babesia* IgM and anti-*Babesia* IgG immunofluorescence assay specific for *B. microti* to confirm the presence of *Babesia* infection. AltNAT1²⁷ (Vitalant Research Institute) detected only *B. microti* and was less sensitive than the Roche in-house validated AltNAT2, which used primers and probes different from cobas Babesia for the detection of the four *Babesia* species.

All donors whose samples were reactive on cobas Babesia were eligible for the follow-up study. Enrolled donors were followed until seroconversion for up to 8 weeks and up to four follow-up visits. Each follow-up collection was tested with cobas Babesia, AltNAT2, anti-*Babesia* IgM, and anti-*Babesia* IgG.

TABLE 1 Limit of detection of *Babesia* infected red blood cells (iRBC) in human whole blood

<i>Babesia</i> species	1.1 ml whole blood input volume				0.4 ml whole blood input volume			
	95% reactive rate (iRBC/mL)	95% confidence range	50% reactive rate (iRBC/mL)	95% confidence range	95% reactive rate (iRBC/mL)	95% confidence range	50% reactive rate (iRBC/mL)	95% confidence range
<i>Babesia microti</i>	2.8	2.3–3.6	0.5	0.5–0.6	6.1	5.0–7.9	1.2	1.1–1.4
<i>Babesia duncani</i>	52.0	45.2–61.7	19.7	18.1–21.4	50.2	44.2–58.8	21.6	20.0–23.4
<i>Babesia divergens</i>	16.3	14.3–19.4	6.6	6.0–7.1	26.1	22.3–31.8	8.2	7.5–9.0
<i>Babesia venatorum</i>	28.3	24.4–34.0	9.6	8.8–10.5	40.0	34.1–48.7	13.0	11.8–14.2

TABLE 2 Limit of detection with *Babesia* RNA transcripts

<i>Babesia</i> species	LOD by Probit analysis			
	95% reactive rate (cp/mL)	95% confidence range	50% reactive rate (cp/mL)	95% confidence range
<i>Babesia microti</i>	38.0	28.9–55.7	7.2	6.1–8.5
<i>Babesia duncani</i>	54.7	41.8–80.5	10.1	8.1–12.0
<i>Babesia divergens</i>	79.7	58.0–124.4	14.2	12.1–16.7
<i>Babesia venatorum</i>	57.8	45.2–81.6	12.5	10.5–14.6

TABLE 3 Testing reactivity patterns and donation status summary—individual donation testing

Reactivity category ^a	Index donation testing				Follow-up study donation testing				Number of donations (total N = 168,981)		
	cobas Babesia	Alt. NAT 1	Alt. NAT 2	Anti-Babesia IgM	Anti-Babesia IgG	Repeat 1:6 pool/R1/R2/R3 cobas Babesia ^b	cobas Babesia ^c	Alt. NAT 2		IgM or IgG positive?	Donation status ^d
1	Reactive	-	+	-	+	(+)/+/+/+	ND	ND	ND	Positive	1
	Reactive	+	+	-	+	(+)/+/+/+	+/+ /+/+	+ /+/+/+	+ /+/+/+	Positive	1
	Reactive	+	+	ND	ND	(+)/+/+/+	+ /+ /ND/ND	+ /+ /ND/ND	+ /+ /ND/ND	Positive	1
	Reactive	+	+	-	+	(+)/+/+/+	+ /- /- /-	+ /+ /- /-	+ /+ /+ /+	Positive	1
	Reactive	+	+	-	-	(+)/+/+/+	ND	ND	ND	Positive	3
	Reactive	+	+	-	+	(+)/+/+/+	ND	ND	ND	Positive	1
	Reactive	+	+	+	+	(+)/+/+/+	ND	ND	ND	Positive	1
3	Reactive	-	-	-	-	(-)/-/-/-	- /- /- /ND	- /- /- /ND	- /- /- /ND	Negative	1
	Reactive	-	-	-	-	(-)/-/-/-	ND	ND	ND	Negative	1
4	Nonreactive	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	168,970

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

Abbreviations: '-', negative/non-reactive; '+', positive/reactive; Alt. NAT, alternative NAT; IgG, immunoglobulin G; IgM, immunoglobulin M; Index donation, donation reactive with cobas Babesia for use with the cobas 6800/8800 Systems; N/A, not applicable; ND, not done; R1/R2/R3, repeat test 1, repeat test 2, repeat test 3.

^aReactivity categories were defined as follows: Category 1—cobas Babesia reactive index donations with positive alternative NAT results (AltNAT1 or AltNAT2). Category 2—cobas Babesia reactive index donation with non-reactive with AltNAT1 or AltNAT2 and positive anti-Babesia IgM or anti-Babesia IgG results (n = 0 donations). Category 3—cobas Babesia reactive index donation with no further reactivity on additional index testing or follow-up testing (i.e., false positive reactive cobas Babesia results). Category 4—donations with non-reactive cobas Babesia results.

^bAdditional cobas Babesia results from simulated pooling (1:6 dilution), if performed, are displayed in parentheses. cobas Babesia results from additional testing of neat replicates, if performed, are displayed with no parentheses in test order separated by a "/".

^cFollow-up study results from up to 4 follow-up visits are displayed separated by a "/".

^dDonation 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.

A reactive result on cobas Babesia was considered a true positive if the index donation or follow-up sample was reactive by either AltNAT, or positive for anti-*Babesia* IgM or anti-*Babesia* IgG. Status negative or uninfected donations were defined as total donations with valid results on cobas Babesia minus true-positive donations. The specificity of cobas Babesia was calculated as the frequency of cobas Babesia nonreactive results among status-negative blood donations for donations overall and separately for donations collected in each of the three *Babesia* endemicity categories: high, low, and nonendemic.

Pooled donations

Donor samples collected between August and November 2019 were tested in pools composed of five or six individual donations at four testing centers. Pooling resolution testing was performed on cobas Babesia reactive pools to determine which donation in the pool was reactive. Each individual reactive sample was then tested as was described earlier for the individual donation study (Figure 2).

All donors whose samples were reactive on cobas Babesia were eligible for the follow-up study. Enrolled donors were followed until seroconversion for up to a 4 weeks and up to two follow-up visits after the date of their index donation. Each follow-up collection was tested as described earlier.

A reactive result on cobas Babesia was considered a true positive if the index donation or follow-up sample was reactive by either AltNAT or positive for anti-*Babesia* IgM or anti-*Babesia* IgG.

The donor-level specificity of the cobas Babesia test was calculated as the percentage of *Babesia* status-negative samples that were nonreactive on the cobas Babesia test for donations overall and separately for donations collected in each of the three *Babesia* endemicity categories. The pool-level specificity of the cobas Babesia test was calculated as the frequency of cobas Babesia nonreactive

pools of six results among pools containing wholly status-negative donations.

The pooled donation study also included the retesting, in pools of 6, samples that were identified as reactive by individual donation screening with cobas Babesia performed during the time of the pooled donation study.

2.3.3 | Pooling deconstruction

A pooling deconstruction study was performed to demonstrate that the sample pooling and functionality of the cobas Synergy Software, together with the Hamilton Microlab STAR for pooling, is capable of identifying *Babesia* NAT reactive specimens in pools of six samples comprised of *Babesia*-positive and *Babesia*-negative specimens.

Nine panels of 96 coded panel members consisting of 813 *Babesia* negative samples and 51 *Babesia* positive samples, representing all four *Babesia* species, were pooled into 16 primary pools of five or six samples and tested with cobas Babesia. The concentrations used for the *Babesia*-positive panel members were such that after a 1:6 dilution, concentrations were approximately 5× LoD. Reactive pools were deconstructed to identify the individual positive samples.

Statistics of overall percent agreement, positive percent agreement (PPA), and negative percent agreement (NPA) between the sample types and deconstruction results were calculated with one-sided 95% lower confidence bounds using the SAS FREQ6 procedure (SAS Institute, Inc.).

3 | RESULTS

3.1 | Analytical sensitivity

For each *Babesia* species, probit analysis on the data combined across dilution series and reagent lots was used to

TABLE 4 Clinical specificity of cobas Babesia—individual donation study

Endemicity	Total number status-negative donations	cobas Babesia result		Estimate in percent (95% exact CI)
		Reactive	Non-reactive	
Overall	168,972	2	168,970	99.999 (99.996, 100.000)
Nonendemic	10,824	0	10,824	100.000 (99.996, 100.000)
Low endemic	14,217	0	14,217	100.000 (99.974, 100.000)
High endemic	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 5 Testing reactivity patterns and donation status summary—pooled donations

Reactivity category ^a	Index donation testing				Follow-up study donation testing					Number of donations (N = 27,729)	
	cobas Babesia result	Alternative NAT 1	Alternative NAT 2	Anti-Babesia IgM +/?	Anti-Babesia IgG +/?	R1/R2/R3 cobas Babesia testing results reactive? ^b	Follow-up study cobas Babesia results reactive? ^c	Follow-up study alternative NAT 2 results reactive? ^c	Follow-up study IgM and IgG positive? ^c		Donation status ^d
1	Reactive	+	ND	+	+	+/?/+	ND	ND	ND	Positive	1
1	Reactive	+	+	+	+	+/?/+	+/?/+	+/?/+	+/?/+	Positive	1
1	Reactive	+	+	-	-	+/?/+	ND	ND	ND	Positive	1
1	Reactive	+	+	+	-	+/?/+	ND	ND	ND	Positive	1
1	Reactive	+	+	+	+	+/?/+	ND	ND	ND	Positive	3
4	Non-Reactive	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	Negative	27,722

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

Abbreviations: -, negative/non-reactive; +, positive/reactive; ?, inconclusive; cobas Babesia, cobas Babesia for use with the cobas 6800/8800 Systems; E, equivocal; IgM, immunoglobulin M; IgG, immunoglobulin G; index donation, donation reactive on cobas Babesia for use with the cobas 6800/8800 Systems; N/A, not applicable; NAT = nucleic acid test; ND, not done; I, invalid.

^aReactivity categories were defined as follows: Category 1—cobas Babesia-reactive index donations with positive alternative NAT 1 and/or alternative NAT 2 results. Category 2—cobas Babesia-reactive index donations with non-reactive alternative NAT (1 & 2) and positive anti-Babesia IgM or anti-Babesia IgG results. Category 3—cobas Babesia-reactive index donations with no further reactivity on additional index testing or follow-up testing (i.e., false-reactive cobas Babesia results). Category 4—Donations with non-reactive cobas Babesia results.

^bcobas Babesia results from additional testing of neat replicates, if performed, are displayed with no parentheses in test order separated by a '/'.
^cFollow-up study results from up to 4 follow-up visits are displayed separated by a '/'.
^dDonation 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.

estimate the 95% and 50% LoD, along with the lower and upper limit of 95% confidence range for *Babesia* iRBC/mL in human whole blood (Table 1) and with *Babesia* transcripts in cp/mL (Table 2).

3.2 | Analytical specificity

All of the microorganism samples with approximately 3× LoD *Babesia* tested reactive and all microorganisms without added *Babesia* tested nonreactive for *Babesia*, indicating that the specificity of cobas Babesia was not affected by the tested microorganisms (Table S1).

3.3 | Inclusivity

All samples of *B. microti* were 100% detected neat and at 4× LoD, and all samples of *B. duncani*, *B. venatorum*, and *B. divergens* cultures were 100% detected at 4× LoD (data not shown).

3.4 | Clinical performance validation/clinical performance evaluation

3.4.1 | Clinical sensitivity

Table S2 shows the clinical sensitivity of the 203 known *Babesia*-positive samples. The overall clinical sensitivity of the clinical and contrived samples across three test sites was 100% (95% CI: 99.4, 100.0) when tested neat (Table S3) and when tested diluted 1:6 (Table S4).

3.4.2 | Clinical specificity

Individual donation studies

Of the 168,981 donations tested by IDT, 143,939 were collected in states with high *Babesia* endemicity, 14,217 in states with low *Babesia* endemicity, and 10,825 in a state where *Babesia* is nonendemic.

Of 168,981 donations, 11 had reactive cobas Babesia results (Table 3). Nine donations were confirmed as true-positives by AltNAT on their index donation; all were reactive on their simulated 1:6 pool results and when retested neat for all three replicates. Five of these were positive for anti-*Babesia* antibody. Two donations were classified as false reactive cobas Babesia results based on additional testing of their index donation or follow-up testing. All nine confirmed positive donations were determined to be *B. microti* on the basis of AltNAT1 or anti-*Babesia* IgG reactivity. Of the positives, 8 were from high endemic states (Minnesota and Wisconsin) and 1 was from a nonendemic state (Iowa).

The clinical specificity of cobas Babesia across all endemicity categories for donations tested individually is 99.999% (95% CI: 99.996, 100.000). The specificity for each endemicity category is shown in Table 4.

Pooled donations

The majority of donations (15,294 of 27,613) tested with cobas Babesia in pools were from high *Babesia* endemicity states, 5834 were from low endemicity states, and 6485 were from non-endemic states.

There were 27,613 donations tested in 4610 pools, including six reactive donations from individual donor screening that were reflexed to the pooled donation study. There were a total of seven reactive pools; each resolved to an individual donation with a reactive cobas Babesia result. All of the reactive individual donations were confirmed positive by AltNAT1 and/or AltNAT2, and all were reactive x 3 by cobas Babesia (Table 5). The seven reactive pools included the six pools containing the donations that had been originally identified by IDT screening; these pools resolved to the IDT-reactive donation. All seven positives were from high endemic states (Minnesota and Wisconsin).

The clinical specificity of cobas Babesia across all endemicity categories for donations tested in pools of six donations is shown in Table 6. There were no false reactive pools (pool specificity 100%, 95% CI: 99.920, 100).

TABLE 6 Clinical specificity of cobas Babesia—pools of 6 (donation level)

Endemicity	Total number of status-negative donations	cobas Babesia reactive	cobas Babesia nonreactive	Estimate in percent (95% exact CI)
Nonendemic	6485	0	6485	100.000 (99.943, 100.000)
Low endemic	5834	0	5834	100.000 (99.937, 100.000)
High endemic	15,287	0	15,287	100.000 (99.976, 100.000)
Overall	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.

Pooling deconstruction

A summary of the positive, negative, and overall percent agreement of the cobas Babesia test with sample type for the deconstruction of the nine panels is shown in Table S5. All 864 samples initially tested in pools of six were correctly identified with an overall agreement of 100% (95% CI: 99.7, 100), 100% PPA (95% CI: 94.3, 100), and 100% NPA (95% CI: 99.6, 100).

4 | DISCUSSION

The performance of the cobas Babesia test in both clinical and nonclinical studies demonstrates its high sensitivity and specificity both by IDT and in pools of six donations. In addition, cobas Babesia can detect all four species of *Babesia* that cause human babesiosis, providing utility not only in the United States but also in other regions where there has been an increase in the detection of *Babesia*.

The analytical sensitivity of *B. microti* at 95% and 50% LoD was 2.8 iRBC/mL (95% CI: 2.3, 3.6) and 0.5 iRBC/mL (95% CI: 0.5, 0.6), respectively, with a sample volume of 1.1 mL in 7.7 mL CPM, which simulates the expected blood sample volume collected in the RWBCT under routine conditions. Taking a conservative approach to account for differences in high altitude that could impact sample collection,²⁴ a sample volume of 0.4 mL was used for the analytical sensitivity claim for the assay. The detection of known positives further supports the sensitivity of cobas Babesia for the detection of all four species of *Babesia* by IDT and in pools of six.

The cobas Babesia assay targets ribosomal RNA (rRNA) as well as DNA. Using infected human clinical samples, Hanron et al. found that *B. microti* rRNA was greater than 1000 times more abundant than coding genes.²⁸ Therefore, each parasite in a donor sample would be expected to release more than 1000 copies of target into the lysate in the RWBCT. Given the high expected target copy number in the lysate from even a single parasite, it is not surprising that in our studies all of the confirmed positive samples detected by individual donation screening were also detectable when diluted 1:6 in a pool or simulated pool. The clinical specificity was excellent for both IDT at 99.999% (95% CI: 99.996, 100.000) and in pools of six at 100% (95% CI: 99.987, 100.000).

In May 2019, the US FDA recommended that blood centers implement year-round regional testing by NAT for blood donations collected in Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, Wisconsin, and Washington, DC.²¹ Pathogen reduction using an FDA-approved device is also allowable; however, it is not currently available for red cells. The cobas

Babesia test was licensed by the US FDA in August 2019 by IDT and for pooling in May 2020; the test is currently in use to screen donations from these endemic regions.

Of the nine confirmed-positive donations detected by IDT, eight were collected in Wisconsin and Minnesota, states considered to be high endemic for *Babesia*. This confirms the presence of *Babesia* in donations in these states which were included in the requirement for testing in the final FDA guidance,²¹ although the prevalence is lower than in the Northeast United States.²⁹ The remaining confirmed-positive donation was collected in Iowa, a *Babesia* nonendemic state. The detection of *Babesia* in a nonendemic state may not be surprising, especially in donors who travel to endemic states. The donor from Iowa was reported to have visited Martha's Vineyard in Massachusetts prior to their donation. A recent analysis by Menis et al. reported an increase of babesiosis in US nonendemic states such as Florida, California, Texas, North Carolina, and Illinois, reinforcing the importance of nationwide monitoring of babesiosis to understand local and travel-associated infections.³⁰ Four of the confirmed positive donations were collected in November, December, January, and March (data not shown). Most cases of babesiosis in the United States occur from late spring to early autumn in endemic areas,¹ although TTB may occur year-round in nonendemic areas,³ supporting the FDA recommendation for year-round testing.

The cobas Babesia test is the first test for the cobas 6800/8800 Systems that utilizes whole blood as the sample type. The ability to use whole blood for NAT is remarkable as heme is a natural inhibitor of polymerase chain reactivity (PCR) by reducing or blocking amplification.³¹ The RWBCT with the addition of a proprietary chaotropic reagent that lyses red blood cells and preserves DNA and RNA has made it possible to minimize the inhibitory effects of heme and provides new opportunities to apply PCR-based technology for the detection of other transfusion-transmitted (TT)-intraerythrocytic pathogens by NAT, such as malaria. The lysate material could be used for multiple tests without having to draw "another tube" from the donor. Another advantage of the RWBCT is that once the sample is received in the laboratory and following centrifugation, no additional steps are required for pipetting or transferring the sample to another test tube prior to testing by IDT; the Whole Blood Collection Tube can be placed directly on the cobas 6800/8800 Systems. This reduction in touchpoints in the preanalytic stage streamlines workflow, reduces opportunities for contamination, and minimizes occupational exposure to bloodborne pathogens. Once the sample is placed on the cobas 6800/8800 Systems, universal sample preparation steps, including nucleic acid extraction and purification, followed by PCR amplification and detection, require minimal operator interaction.

In summary, the performance of the cobas Babesia test for use on the automated cobas 6800/8800 Systems demonstrates excellent sensitivity and specificity for the detection of all four species of *Babesia* known to cause human infection.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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