



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Biostatistics and Epidemiology
Division of Biostatistics

Statistical Review and Evaluation BLA (Final)

Product Name:	Babesia microti Nucleic Acid Test (NAT- STN125588/0) and Babesia microti Arrayed Fluorescence Immunoassay (AFIA- STN125589/0)
Indication(s):	<ul style="list-style-type: none">• Babesia microti NAT test is intended for use as a donor screening test to detect B. microti DNA in whole blood samples from individual human donors, including volunteer donors of whole blood and blood components, as well as other living donors (STN125588).• Babesia microti Arrayed Fluorescence Immunoassay (AFIA) test is intended for use as a donor screening test to detect antibodies to B. microti in (b) (4) plasma samples from individual human donors, including volunteer donors of whole blood and blood components, as well as other living donors (STN125589).
Applicant:	IMUGEN, Inc.
Receipt Date(s):	5/12/2015 (original submission) 12/14/2016 (resubmitted, response to CR) 10/20/2017 (resubmitted, response to 2 nd CR)
Review Priority:	Priority review
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1 . Executive Summary

Babesiosis is an infection caused by *Babesia*, which can be transmitted to humans through blood transfusion products derived from *Babesia* infected donors. Two submissions from the same applicant were submitted together: one for *Babesia microti* Polymerase chain reaction (PCR) assay (BLA125588) and the other one for indirect fluorescent antibody (IFA) assay (BLA125589). Both are covered in this review.

FDA issued a complete response (CR) letter on September 29, 2015, due to the absence of clinical sensitivity study and many other deficiencies. After being granted for an extension of time to respond, a complete response to the CR letter and updated results were received on December 14, 2016, which are the main focus of this review. A second CR letter was sent on June 13, 2017 due to non-statistical issues.

Based on the algorithm for calculation, both assays reached high specificities (99.95% -100% for point estimate and 99.86%-99.99% for lower bound of 95% CI) in the donor population (non-endemic or endemic region) and high sensitivity (100%) in the known positive samples. The results are verified. There are no remaining statistical issues.

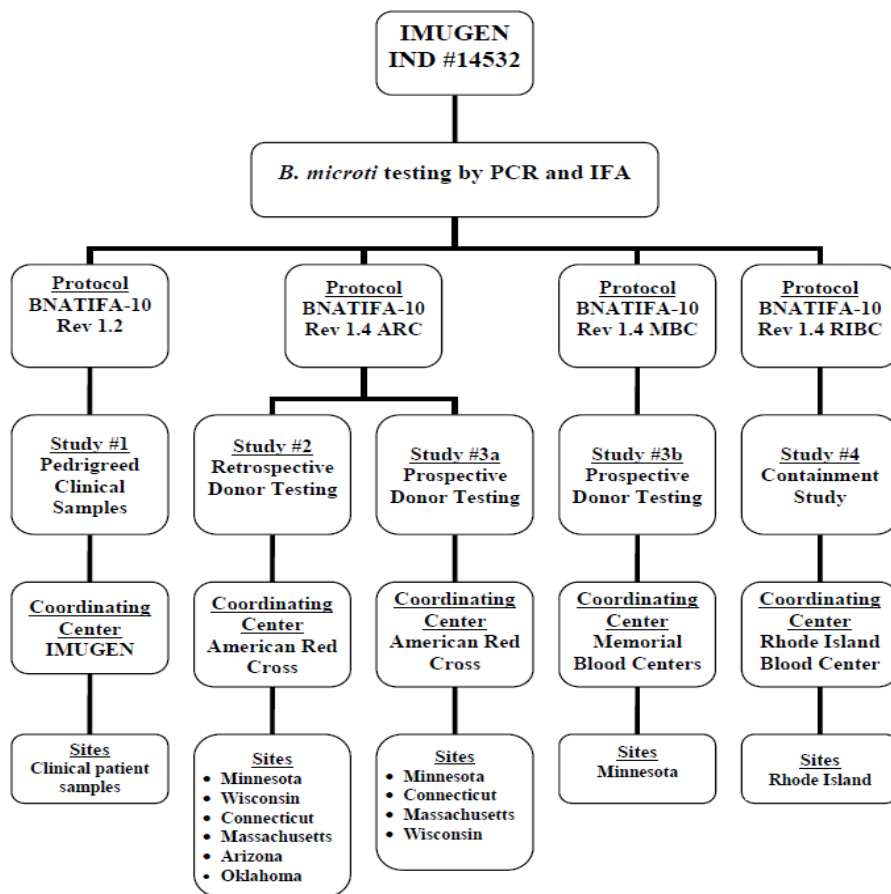
2 . Background

Babesiosis is an infection caused by intra-erythrocytic protozoa of the genus *Babesia*. The most common specie causing disease in the United States is *B. microti*. *Babesia* can be transmitted to humans through blood transfusion products derived from *Babesia* infected donors.

Currently, there are no FDA licensed tests for the clinical diagnosis or blood donor screening of the babesiosis infection. On May 12, 2015, Imugen, Inc. submitted two BLAs at the same time for *Babesia microti*, Polymerase chain reaction (PCR), and indirect fluorescent antibody (IFIA) assays (referred to as the "NAT (nucleic acid test) - BLA125588" and "AFIA Test - BLA125589," respectively).

Five studies were conducted under IND14532 as outlined in Figure 1. Study 1 was an exploratory study to evaluate the clinical significance of different cut-off values, which was more appropriately identified as a pre-clinical study and no assessment was performed. Study 2 was an investigational screening for *Babesia microti* in a large repository of blood donor samples from non-endemic and endemic areas of the United States. Study 3a was designed to test prospective blood donors for evidence of *B. microti* infection. Blood donations were collected at American Red Cross (ARC) blood drives and collection sites across the high, low-medium and non-endemic regions in USA. Study 3b was a small, prospective study conducted only in Minnesota (a low endemic area). Study 4 was a containment study to reduce or eliminate transfusion transmitted Babesia in selected patient recipient populations (e.g. neonates, sickle cell, thalassemia, asplenic, pediatric, etc.); the study was conducted at Rhode Island Blood Center (RIBC).

Figure 1: General Investigational Plan Flow Chart



Source: Figure 8.4.2.1, page 13 of 33 of Attachment 2.3.

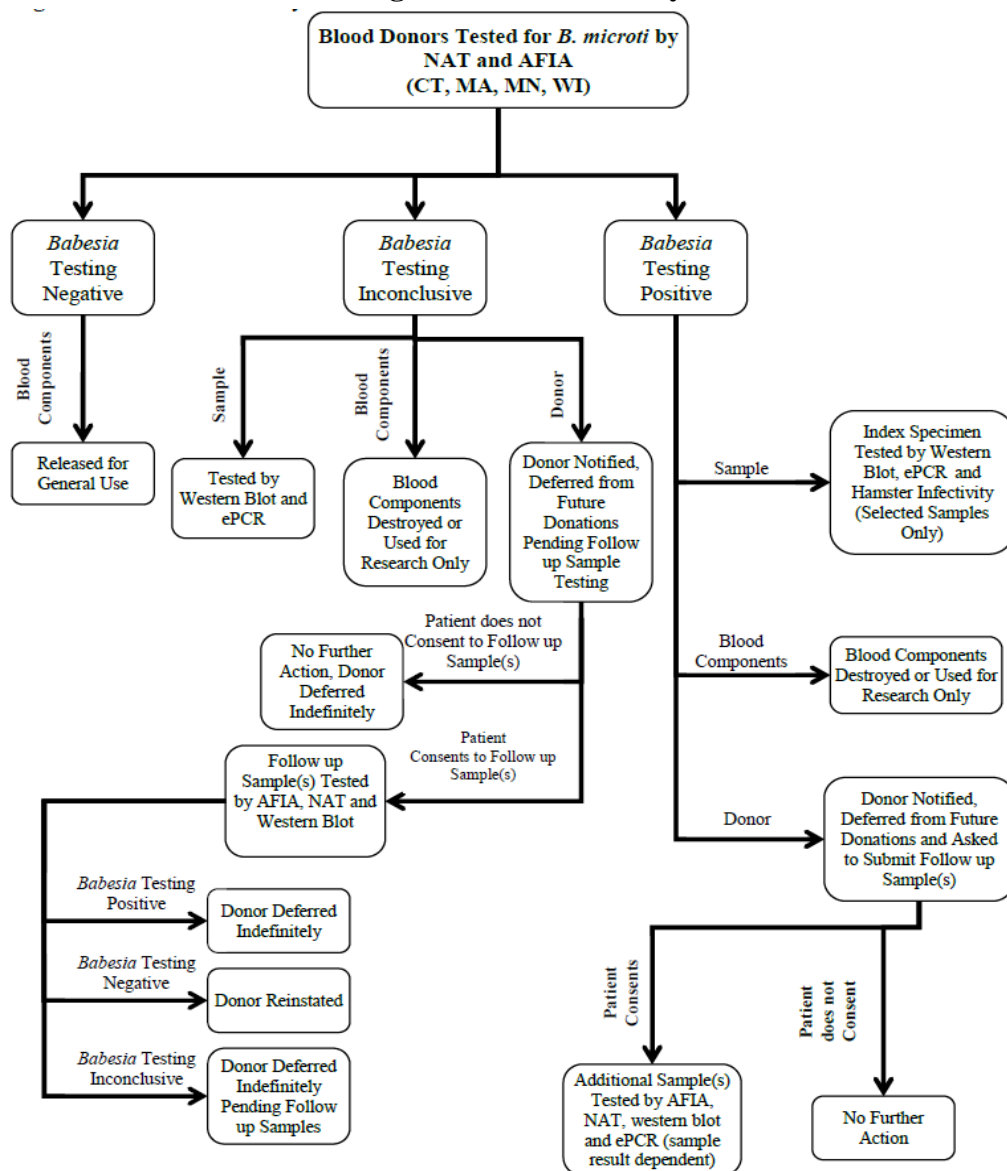
Among the five studies, Studies 2, 3a, 3b, and 4 will be reviewed in Section 3.

The submission also included two other studies: sensitivity and precision studies. The sensitivity study was not included in the original submission, although it was requested so in the clinical hold letter to IND 14532 dated December 10, 2010. Per FDA’s request in the CR letter dated September 29, 2015, the results of sensitivity study for both investigational products were submitted on December 14, 2016. The analysis of precision and reproducibility studies, provided in the original submission, was not appropriate. Per FDA’s request in the CR letter, the revised analysis result was submitted on December 14, 2016. A second CR letter was sent on June 13, 2017 due to non-statistical issues.

3. Study Performance Evaluation

For the studies in Figure 1, the overall study flow is presented in Figure 2. Please note that “Babesia Testing Positive” could be positive by either assay (NAT or AFIA) and “Babesia Testing Negative” required a negative result by both assays.

Figure 2: Overall Study Flow



Source: Figure 8.4.3.2, page 17 of 63 of Attachment 2.4

The retest procedure and final interpretation of NAT and AIFA results are summarized below.

a) ***B. microti* NAT by PCR**

All NAT-positive samples were retested in (b) (4) (see Table 1).

Table 1: NAT results interpretation

Initial Result	Repeat Rep	Repeat Rep	Repeat Rep	Interpretation	Reported Result
Negative	Not re-tested			No <i>B. microti</i> DNA detected.	Negative
Positive	Negative	Negative	Negative	<i>B. microti</i> DNA <u>cannot be confirmed</u> .	Inconclusive ¹
Positive	1 or more positive replicates			<i>B. microti</i> DNA detected	Positive

¹A non-repeating NAT positive is considered “Inconclusive”. The concentration of *Babesia* may be below the nominal probability of detection

Source: Table 8.4.3.4, page 21 of 63 of Attachment 2.4

b) *B. microti* Antibody Testing by AFIA

All AFIA positive specimens were retested at the screening dilution of 1:128 and were titrated in two-fold increments (128-1024) to determine an endpoint titer. The results from initial and repeat testing (n=2) for the Babesia AFIA assay were interpreted as in Table 2.

Table 2: AFIA Results Interpretation

Initial Result	Repeat Rep	Repeat Rep	Interpretation	Reported Result
Negative	Not re-tested	Not re-tested	<u>No</u> <i>B. microti</i> antibody detected	Negative
NSF	Negative	Negative	<u>No</u> <i>B. microti</i> antibody detected	Negative
NSF	1 or more NSF replicates		<i>B. microti</i> antibody status <u>cannot be determined</u> .	Inconclusive
NSF	Negative	Positive	<i>B. microti</i> antibody status <u>cannot be determined</u> .	Inconclusive
NSF	Positive	Positive	<i>B. microti</i> antibody detected.	Positive
Positive	Negative	Negative	<u>No</u> <i>B. microti</i> antibody detected	Negative
Positive	Negative	NSF	<i>B. microti</i> antibody status <u>cannot be determined</u> .	Inconclusive
Positive	NSF	NSF	<i>B. microti</i> antibody status <u>cannot be determined</u> .	Inconclusive
Positive	1 or more positive replicates		<i>B. microti</i> antibody detected.	Positive

NSF = non-specific fluorescence, considered “Inconclusive” since antibody status cannot be determined.

Source: Table 8.4.3.5, page 22 of 63 of Attachment 2.4

Specificity Calculations:

Specificity was calculated according to the formula below:

NAT:

- Unconfirmed NAT positives = NAT positive donors whose index and follow-up specimens are AFIA negative.
- True Negatives = Index specimens that are NAT negative

% Specificity = # True Negatives ÷ (# True Negatives + Unconfirmed Positives) x 100,

AFIA:

- Unconfirmed AFIA positives = AFIA positive index specimens that are NAT negative and Western Bolt (WB) negative.
- True Negatives = Index specimens that are AFIA negative

The two-sided 95% confidence intervals (CI) of specificity were also calculated. Please note that specificity was only calculated in Study 2 and Study 3a.

3.1 Study 2: Retrospective ARC Study

3.1.1 Study Objectives

- Determine the frequency of *Babesia* positive findings in high-endemic, low-medium endemic and non-endemic regions.
- Determine the specificity of the AFIA and NAT assays.

3.1.2 Study Design

The target was to screen 13,000 repository blood samples for *B. microti* by IMUGEN's AFIA and NAT assays, following the overall study flow (Figure 2). All samples were collected in the months of May-September in 2010 and 2011.

3.1.3 Result:

A total of 373 repository specimens were excluded from the study, primarily (n=367) due to clotting. In addition, 77 samples have been removed from analysis per FDA's recommendation in the NAT CR letter Question #2. A total of 13,192 repository blood samples were included in the final analysis.

The donor testing results are summarized in Table 3 (AFIA cutoff 1:128) by state. The frequency of *Babesia* positive donors was 0.55% (28/5,059) in the predicted high endemic region (Connecticut and Massachusetts), 0.07% (3/4,164) in the predicted low-medium endemic region (Minnesota and Wisconsin), and 0.03% (1/3,969) in the predicted non-endemic region (Arizona and Oklahoma). The state of Connecticut had the highest frequency of *Babesia* positive findings of 1.34% (24/1,783).

Table 3: Donor Testing Results Summary by State (NAT and AFIA (1:128)) in Study 2

State	Number of Donors Screened	NAT POS	AFIA POS	NAT INC	AFIA INC	TOTAL POS DONOR (NAT and/or AFIA)	TOTAL POS/INC DONORS
		n	n	n	n	n (%)	n (%)
Connecticut	1783	4	24	0	0	24 (1.35)	24 (1.35)
Massachusetts	3276	1	4	0	1	4 (0.12)	5 (0.15)
Minnesota	2041	1	1	0	0	1 (0.05)	1 (0.05)
Wisconsin	2123	1	2	1	1	2 (0.09)	4 (0.19)
Arizona	2000	0	0	0	0	0 (0)	0
Oklahoma	1969	0	1	0	0	1 (0.05)	1 (0.05)
Total	13192	7	32	1	2	32 (0.24)	35 (0.27)

Source: Created by this reviewer based on the updated data line

- **Specificity in Non-Endemic Region (AZ and OK):**

Assuming the non-endemic specimens were all true negative for antibodies to *Babesia microti*; there was 1 AFIA positive (with the cutoff 1:128) donor and no AFIA inconclusive donors out of 3,969 donors in the non-endemic region. No NAT positive and inconclusive was observed.

NAT Specificity: $3,969 \div (3,969 + 0) = 100\%$, 95% CI (99.91%, 100.00%)

AFIA Specificity: $3,968 \div (3,968 + 1) = 99.97\%$, 95% CI (99.86%, 100.00%)

- **Specificity in Endemic Region (CT, MA, MN, WI):**

Table 4: NAT vs. AFIA in Study 2

	AFIA (≥ 128 Cutoff)			Total
	Positive	Inconclusive	Negative	
NAT Positive	7	0	0	7
NAT Inconclusive	0	0	1	1
NAT Negative	24	2	9189	9215
Total	31	2	9190	9223

Source: Table 8.4.2.17 Page 29 of 33, Attachment 2.3

NAT Specificity: $9,189 / (9,189 + 1) = 99.99\%$, 95% CI (99.94%, 100.00%)

Table 5: AFIA vs. NAT and WB using Endemic Data in Study 2

	NAT or WB Positive	NAT or WB Inconclusive	NAT and WB Negative	Total
AFIA Positive	31	0	0	31
AFIA Inconclusive	0	0	2	2
AFIA Negative	11 ⁱ	1	9178 ⁱⁱ	9190
Total	42	1	9180	9223

i. All 11 specimens tested positive at a 1:64 dilution and negative at a 1:128 dilution and are therefore considered AFIA negative for purposes of this analysis

ii. WB not performed on specimens testing NAT negative and AFIA negative

Source: Table 8.4.2.16 Page 28 of 33, Attachment 2.3

AFIA Specificity: $9,178 \div (9,178 + 2) = 99.98\%$, 95% CI (99.92%, 100.00%)

3.2 Study 3a: Prospective ARC Study

3.2.1 Study Objectives

- Determine the performance characteristics of *B. microti* NAT and AFIA assays by testing prospective blood donor samples.
- Determine the seasonal incidence/prevalence of *B. microti* antibody (AFIA) and/or NAT positives in the donor populations of *B. microti* endemic and non-endemic areas.
- Correlate AFIA and NAT results.

3.2.2 Study Design

Blood donations were collected at ARC from regions of predicted varying endemicity for *B. microti*.

All blood or cellular components with donor status interpreted as inconclusive or positive by either AFIA and/or NAT would not be used for transfusion purposes. Donors with “inconclusive” status would be asked to return for follow-up testing in ≥ 8 weeks.

3.2.3 Result:

A total of 88,904 blood donor specimens were screened. The dates of collection and numbers of sample analyzed by region are displayed in Table 6.

Table 6: Clinical Study Blood Donors Distribution by Region in Study 3a

Predicted Endemicity	Geographical Area	Target Number of Donors	Number of Donors Screened	Dates of Collection
High Endemic Area	Connecticut (CT) Massachusetts (MA)	4,000	75,082	6/4/2012- 9/30/2014
Low- Medium Endemic Area	Minnesota (MN)	2,000	13,822	2/4/2013- 9/30/2014
	Wisconsin (WI)	2,000		
Non-endemic Area	Location not determined ⁱⁱ	2,000 – 4,000	0	
Total	All Areas	10,000-12,000	88,904	6/4/2012- 9/30/2014

ii: American Red Cross was unable to provide prospective samples from non-endemic regions. Retrospective samples from the non-endemic regions of AZ and OK were obtained and tested, and included in Study #2 (Retrospective Blood Donor Testing).

Source: Table 8.4.3.7, page 27 of 63, Attachment 2.4

A total of 809 donor specimens were unsuitable for testing and therefore were excluded from the study. The reasons of exclusion are summarized in Table 7.

Table 7: Reasons of Exclusion in Study 3a

Status	MA	CT	MN	WI	TOTAL
Empty	26	1	0	0	27
QNS	258	486	5	24	773
Other ⁱ	7	2	0	0	9
NAT Complete Response Question #2	106	143	0	0	249
Total	291	489	5	24	809

i: Tube broken, leaking sample or unlabeled sample; QNS: quantity not sufficient

Source: Table 8.4.3.8, page 27 of 63, Attachment 2.4

There were 338 (0.38%) *Babesia* positive and 2 (0.002%) *Babesia* inconclusive donors identified from 88,904 donor units tested in Study 3a. These findings were classified by region (states) and presented in Table 8; it showed that the Geographic Distribution frequency of *Babesia* positive donors was 0.43% (325/75,082) in the high endemic region (Connecticut and Massachusetts) and 0.09% (13/13,822) in the low-medium endemic region (Minnesota and Wisconsin).

Table 8: Overall Study Results in Study 3a

State	Total # of Donor Samples	NAT Positive N(%)	AFIA Positive N(%)	Total Positive Donor Samples (NAT and/or AFIA) N(%)
Connecticut	38,922	46 (0.12%)	246* (0.63%)	251 (0.64%)
Massachusetts	36,160	13** (0.04%)	71* (0.20%)	74 (0.20%)
Minnesota	11,726	2 (0.02%)	9 (0.08%)	10 (0.09%)
Wisconsin	2,096	1 (0.05%)	3 (0.14%)	3 (0.14%)
State Totals	88,904	62 (0.07%)	329 (0.37%)	338 (0.38%)

* 2 AFIA Inconclusive results, 1 from both CT and MA, are not included in the table.

** 1 NAT Inconclusive result from MA is not included in the table.

Source: Table 8.4.3.9 page 28 of 63, Attachment 2.4

The frequency of *Babesia* positive donors by tick transmission “season” for the total study population in each geographic region is shown in Table 9.

Table 9: *Babesia* Positive Donor Frequency by Transmission “Season” in Study 3a (NAT and AFIA Combined)

	State or States	MAY-NOV n (%)	DEC-APR n (%)	p-value
High Endemic	CT	171/28,156 (0.61%)	80/10,766 (0.74%)	0.1580
	MA	47/24,503 (0.19%)	27/11,657 (0.23%)	0.4559
	CT/MA Combined	218/52,659 (0.41%)	107/22,423 (0.48%)	0.2500
Low-Medium Endemic	WI/MN Combined	9/9818 (0.09%)	4/4004 (0.1%)	0.8861
	Total All States	227/62,477 (0.36%)	111/26,427 (0.42%)	0.2305

Source: Table 8.4.3.12 page 30 of 63, Attachment 2.4

Specificity Result:

As described before, the true status to calculate the specificity of NAT was based on index and follow-up AFIA results, while it was determined by NAT and WB for the specificity calculation of AFIA.

The specificity of NAT is shown in Table 10.

Table 10: Specificity of NAT assay vs. AFIA at index and/or follow-up in Study 3a

	AFIA (+)	AFIA (Inc)	AFIA (-)	TOTAL
NAT (+)	60 ⁱ	0	1 ^{ii, iii}	61
NAT (Inc)	1	0	0	1
NAT (-)	275	2	88564	88841
TOTAL	336	2	88565	88903

(i) Donors testing AFIA positive at index or in a follow up sample; (ii) Donors testing AFIA negative at index and in all follow up samples; (iii) Excludes 1 donor whose index sample was NAT positive and AFIA negative with no follow up sample available.

Source: Table 8.4.3.41 page 56 of 63, Attachment 2.4

NAT Specificity: $88,564 / (88,564 + 1) = 99.999\%$, 95% CI (99.99%, 100.00%)

There were 9 window period donors, NAT positive and AFIA negative at time of screening, identified in the study, and 8 had at least one follow-up sample available for analysis. Among the 8 window period donors, 7 had evidence of seroconversion by AFIA and WB.

There was one NAT inconclusive result (Donor (b) (6) from Massachusetts). This donor was AFIA positive, WB positive and ePCR positive at index. Hamster infectivity was not performed. A follow-up sample at 27 days after index was NAT negative, ePCR positive, with ongoing positive antibody results.

The specificity of AFIA is shown in Table 11.

Table 11: Specificity of AFIA Assay vs. NAT and WB in Study 3a

	NAT and/or WB (+)	NAT and/or WB (Inc) ⁱ	NAT and WB (-)	TOTAL
AFIA (+)	291	3	33 ⁱⁱ	327
AFIA (INC)	0	0	2	2
AFIA (-)	9	0	88,564 ⁱⁱⁱ	88,573
TOTAL	300	3	88,599	89,902

Note: Two QNS, one specimen had insufficient volume (QNS) for AFIA and one smear result was clotted and not interpretable, were deleted from the analysis.

Source: Table 8.4.3.40 page 55 of 63, Attachment 2.4

AFIA Specificity: $88,564 / (88,564 + 35) = 99.96\%$, 95% CI (99.95%, 99.97%)

There were 2 AFIA “inconclusive” results. One donor was AFIA inconclusive at index without evidence of antibody by WB and did not have evidence of *Babesia* by NAT or ePCR. The other donor was found to be AFIA positive and WB positive at the first follow-up sample obtained 165 days post index. Follow-ups obtained at 277, 373 and 467 days post index were AFIA positive and WB negative, and the donor became AFIA negative/WB negative at the final follow-up 550 days post-index.

3.3 Study 3b:

Study 3b was a small, prospective study conducted only in Minnesota (a low endemic area). A total of 1,187 whole blood units were screened prospectively for *B. microti*. There were no positive or inconclusive findings identified in this study by either NAT or AFIA. No cases of

transfusion transmitted babesiosis were reported or documented from any screened units of blood in this study.

3.4 Study 4:

Clinical Study 4 was a containment study to reduce or eliminate transfusion transmitted *Babesia* in selected patient recipient populations (e.g. neonates, sickle cell, thalassemia, asplenic, pediatric, etc.). Study 4 was conducted at Rhode Island Blood Center (RIBC) with a total of 3,682 whole blood units screened prospectively for *B. microti*. Seven positive donor units were identified by either NAT or AFIA (titer >128); one sample was both NAT and AFIA positive, and six samples were only AFIA positive. There were no inconclusive results identified in this study.

3.5 Clinical Sensitivity:

3.5.1 NAT clinical sensitivity study:

The sensitivity of the investigational NAT assay was determined by testing 72 blood smear positive specimens which was planned to obtain a lower limit of 95% CI of sensitivity > 95%. In addition, 23 smear negative specimens were tested to address potential bias in testing. The negative specimens were randomly interspersed within the total test group.

Result: All the 72 *Babesia microti* blood-film-positive samples were positive by NAT, with 95% CI (95.01%, 100%). Twenty-two (22) of the 23 negative specimens were negative by NAT, one was inconclusive. The inconclusive result was obtained at high CT value; it was tested negative upon repeat.

3.5.2 AFIA clinical sensitivity study:

Seventy-two (72) blood-film confirmed *Babesia* infected samples were included in the study to obtain a lower Confidence Interval (CI) of > 95%. In addition, the study included 20 *Babesia* infection negative samples. The negative specimens were randomly interspersed within the total test group. Sensitivity was established from the AFIA assay outcomes for the samples according to the algorithm with positives = ≥ 128 ; negative, otherwise.

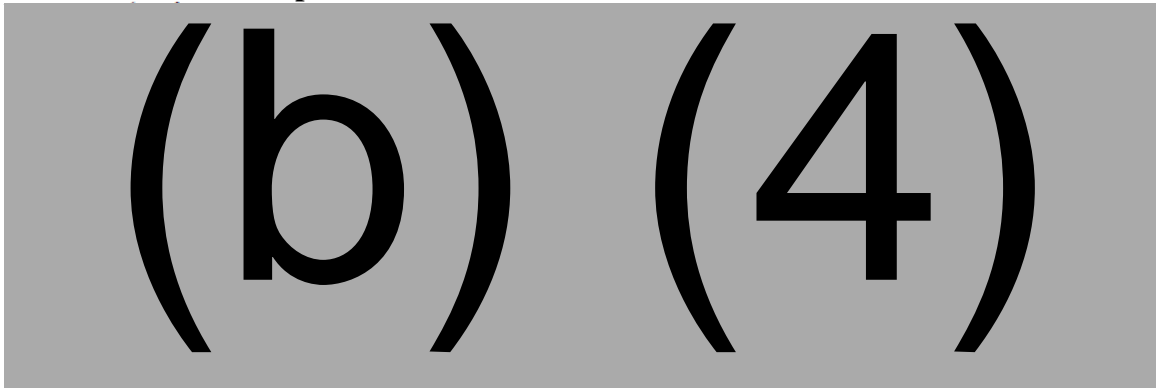
Result: All the 72 *Babesia microti* blood-film positive samples were AFIA positive, with 95% CI (95.01%, 100.00%). All the 20 *Babesia microti* negative samples were AFIA negative.

3.6 Reproducibility and Precision Study for NAT

3.6.1 Study Design

Study specimens consisted of negative human whole blood spiked with positive control blood in a panel consisting of three test specimens: (1) a “weak negative” at $(b) (4)$ LOD $(b) (4)$ copies per mL, (2), a sample at $(b) (4)$ LOD $(b) (4)$ copies/mL, and (3) a “weak positive” formulated at $(b) (4)$ LOD $(b) (4)$ copies/mL. In these three panels, $(b) (4)$ samples were extracted from each specimen. And, each sample was tested in a $(b) (4)$ procedure: $(b) (4)$ runs per day, $(b) (4)$ replicates per run for $(b) (4)$ days (total $(b) (4)$ data points). The other panels included the No Template, Negative Control, Low Positive Control and High Positive Control; a sample extracted in each control was tested $(b) (4)$ runs per day, $(b) (4)$ per run for over $(b) (4)$ days (total $(b) (4)$ data points). Testing was summarized in Table 12.

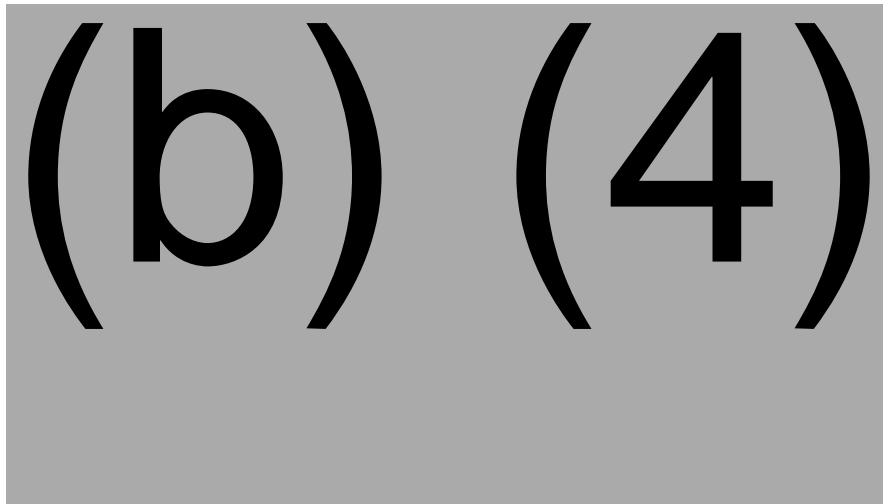
Table 12: Expected Results and Total N for NAT Precision Studies



The applicant indicated that the precision testing would challenge the (b) (4) sources of potential variability: (1) the (b) (4) and reactive reagents, N = (b) (4) lots, (2) the extraction kit (N = (b) (4) lots) (3) the (b) (4) extractor (N = (b) (4) systems) (4) the (b) (4) (N = (b) (4) instruments) and (5) Operators (N = (b) (4)). Studies were performed using the established *Babesia* NAT procedures.

3.6.2 Statistical Method and Acceptance Criteria:

The results were analyzed in two ways: qualitatively based on agreement and quantitatively using Ct values with SD and %CV provided. The acceptance criteria of qualitative assessment are shown in Table 13. For quantitative analysis, the %CVs should be within acceptable limits (b) (4)



3.6.3 Results:

In the qualitative assessment, except for the panel of (b) (4) LOD that (b) (4) negative result was observed, all other panels met all acceptance criteria for all samples (see Table 13).

For the quantitative analysis, the precision study reports provided in the submission included (1) Summary of Overall Results (2) Results by Lot (3) Results by Run/Operator (4) Results by Instrument. The Summary of Overall Results is presented in Table 14.

(b) (4)

3.7 Reproducibility and Precision Study for AFIA

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

4. Final Conclusions:

The study results are summarized as below:

- **Specificity in Non-Endemic Region, Study 2 (AZ and OK):**

NAT : 100%, with 95% CI (99.91%, 100.00%)

AFIA: 99.97% with 95% CI (99.86%, 100.00%)

- **Specificity in Endemic Region, Study 2 (CT, MA, MN, WI):**

NAT : 99.99%, with 95% CI (99.94%, 100.00%)

AFIA: 99.98%, with 95% CI (99.92%, 100.00%)

- **Specificity in Prospective ARC Study, Study 3a**

NAT : 99.99%, with 95% CI (99.99%, 100.00%)

AFIA: 99.96%, with 95% CI (99.95%, 99.97%)

- **Clinical Sensitivity Study:**

NAT and AFIA:100%, with 95% CI (95.01%, 100.00%).

- **Reproducibility and Precision Study**

NAT: In the qualitative assessment, except for the panel of (b) (4) LOD that (b) (4) negative result was observed, all other panels met all acceptance criteria for all samples.

AFIA: There was no error in all (b) (4) tested, and all tests gave 100% expected results.

I verified the above results.