Summary Basis for Regulatory Action

Date: March 06, 2018

From: Babita Mahajan, Chair of the Review Committee

BLA/ STN#: 125588

Applicant Name: Oxford Immunotec Ltd.

Date of Submission: May 12, 2015

Complete Response Letter 1: September 29, 2015

Resubmission 1: December 14, 2016

Complete Response Letter 2: June 13, 2017

Resubmission 2: October 10, 2017

MDUFA Goal Date: April 11, 2018

Proprietary Name: Imugen *Babesia microti* Nucleic Acid Test (NAT)

Established Name (common or usual name): *Babesia microti* NAT/*Babesia microti* NAT for Blood Screening

Intended Use/Indications for Use:

The Imugen *Babesia microti* NAT is a nucleic acid screening assay for the detection of *Babesia microti* DNA in human whole blood samples (with EDTA as anti-coagulant).

This test is intended for use as a donor screening test to detect *Babesia 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. It is also intended for use to screen organ and tissue donors when specimens are obtained while the donor's heart is still beating.

This test is not intended for use on specimens from cadaveric (non-heart beating) donors.

The test is not intended for use on samples of cord blood.

This test is not intended for a use as an aid to diagnosis of *Babesia microti* infection.

Recommended Action: The Review Committee recommends approval of this product.

Review Office Signatory Authority: Nicole Verdun, M.D., Acting Director, OBRR/CBER

□ I concur with the summary review.
□ I concur with the summary review and include a separate review to add further analysis.
□ I do not concur with the summary review and include a separate review.

Office of Compliance and Biologics Quality Signatory Authority: Mary A. Malarkey, Director, OCBQ/CBER

□ I concur with the summary review.
□ I concur with the summary review and include a separate review to add further analysis.
□ I do not concur with the summary review and include a separate review.

The table below indicates the material reviewed when developing the SBRA.

Table 1: Reviews Submitted

Document Title	Reviewer Name	Document Date
Product Review (DETTD) *Clinical* *Non-Clinical*	 Pawan Jain Luisa Gregori Rana Nagarkatti Robert Duncan	 Apr 18, 2017 Jan 10, 2018 Jun 1, 2017 Jan 31, 2018
Statistical Review *Clinical* *Non-Clinical*	 Paul Hshieh	 Dec 29, 2017
CMC Review *CMC (DETTD)* Facilities Review *(OCBQ/DMPQ)* Establishment Inspection *Report (OCBQ/DMPQ)*	 Erica Silberstein Ranadhir Dey Sreenivas Gannavaram Lori Peters Lori Peters Sean Byrd Justine Corson (ORA) Babita Mahajan (DETTD) Robert Duncan (DETTD)	 Dec 5, 2017 Dec 7, 2017 Feb 15, 2018 Feb 12, 2018 Feb 12, 2018
Labeling Review *APLB (OCBQ/APLB)* *Product Office*	 Dana Jones Babita Mahajan	 Sep 9, 2015
Lot Release Protocols/ Testing Plans	Marie Anderson Kori Francis	Dec 12, 2017 Feb 12, 2018
Bioresearch Monitoring Review	Bhanu Kannan	Dec 2, 2015
Software and Instrumentation	Lisa Simone Yongqing Chen	Feb 7, 2018 Feb 15, 2018

1. Introduction

IMUGEN, Inc., located at 315 Norwood Park South, Norwood, MA, submitted an original Biologics License Application (BLA) for licensure of the *Babesia microti* Nucleic Acid Test (NAT). This is the first NAT based blood donor screening assay for *B. microti* parasites. The BLA was granted priority review status based on an unmet public health need due to the lack of a screening test for whole blood donations and the expanding incidence of *B. microti* infections from the Northeast and upper Midwest to the Mid-Atlantic regions of the United States. The Imugen *Babesia microti* Nucleic Acid Test is an "in-house" test performed only by the sponsor; and no kits are sold.

This BLA application from IMUGEN, Inc. was received on May 12, 2015 as a paper submission with electronic content (DCC login 607587). This submission was filed on

July 10, 2015 and the mid-cycle meeting was held on August 17, 2015. A Complete Response (CR) Letter was issued on September 29, 2015. On July 1, 2016, FDA was informed of an ownership change for BLA 125588 from IMUGEN, Inc. to Oxford Immunotec Ltd. On September 16, 2016, FDA received an amendment from the sponsor requesting an extension of 6 months for its response to FDA's CR Letter dated September 29, 2015. The response to the CR Letter was submitted on December 14, 2016 and the amendment was classified as Class 2 resubmission. An Information Request (IR) Letter was sent on February 17, 2017. CBER conducted an establishment Pre-License Inspection (PLI) of the Oxford Immunotec Inc., d/b/a Imugen (hereinafter referred to as "Imugen") facility from March 6 through 10, 2017. FDA noted serious concerns at the end of the inspection that were conveyed to the sponsor in the form of observations on FDA Form 483. The sponsor responded to the FDA Form 483 on April 17, 2017, and it was concluded that the sponsor didn't sufficiently address the concerns noted during the inspection. Additionally, the sponsor had not responded and resolved the software and instrumentation deficiencies. A second CR Letter was issued on June 13, 2017. Two submission issue meetings were requested to discuss 483 inspection issues (BQ170068) and software issues (BQ170083), however, the sponsor was satisfied with the written responses provided by the review committee and the meetings were cancelled. The response to the CR Letter was submitted on October 10, 2017 and the amendment was classified as a Class 2 resubmission.

Concurrent Submission
BL125589: Imugen *Babesia microti* Arrayed Fluorescence Immunoassay (AFIA) – Received May 12, 2015

Table 2: Chronological Summary of Submission and FDA Correspondence

Date	Action	Amendment to BL125588
May 12, 2015	BLA CBER receipt	
May 19, 2015	Priority request	
Jun 5, 2015	Acknowledgement Letter	
Jun 12, 2015	Updated summary	/0/1
Jul 03, 2015	Updated Master validation information	/0/4
Jul 10, 2015	Filing Notification Letter	
Sep 23, 2015	Lot Release Protocol	/0/26
Sep 29, 2015	Complete Response Letter	
Jul 01, 2016	Notice of change in BLA ownership; IMUGEN, Inc. was acquired by Oxford Immunotec Ltd.	
Sep 16, 2016	Request for extension to respond to CR	/0/11
Dec 14, 2016	Response to CR Letter; Resets the goal date to Jun 14, 2017	/0/13
Feb 17, 2017	FDA IR on CR responses	
Mar 01, 2017	Partial response to IR	/0/14
Mar 6-10, 2017	Pre-license inspection; FORM FDA 483 issued	
Mar 7, 2017	Change in BLA ownership filed	/0/16

Mar 23, 2017	Complete response to IR	/0/15
Apr 5, 2017	FDA IR on Lot release template	
Apr 13, 2017	Request for face-to-face meeting on manufacturing scale up (BQ170100)	/0/18
Apr 14, 2017	FDA IR on software	
Apr 17, 2017	483 Response	/0/17
Apr 20, 2017	Teleconference to discuss software issues	
May 3, 2017	Sponsor meeting packet	/0/20
May 5, 2017	Sponsor submission of Lot Release Template	/0/19
May 10, 2017	Face to Face Meeting to discuss manufacturing scale-up	BQ170100
May 18, 2017	Call request to discuss software issues	/0/22
May 18, 2017	483 Resolution Progress	0/23
May 18, 2017	Sponsor changes point of contact	/0/24
May 23, 2017	Sponsor response to IR (software)	/0/25
Jun 5, 2017	483 Resolution Progress	/0/27
Jun 13, 2017	483 Resolution Progress	/0/28, 29
Jun 13, 2017	FDA Complete Response Letter #2	
Aug 21, 2017	Submission issue meeting - written response to 483 issues	BQ170093
Oct 10, 2017	Response to CR#2; Resets the goal date to Apr 11, 2018	/0/32
Oct 26, 2017	Updated Lot release template	/0/33
Nov 9, 2017	FDA IR for software issues	
Nov 20, 2017	Response to software IR	/0/34
Dec 1, 2017	Interactive review of performance data; Lot release testing	/0/35, 36
Dec 15, 2017	Information request – DMPQ 483 issues	
Dec 18, 2017	Teleconference (DMPQ)	
Jan 9, 2018	Response to DMPQ IR	/0/37
Jan 29, 2017	Interactive review of performance data	/0/38
Feb 2, 2018	FDA IR for DMPQ 483 issues	
Feb 6, 2018	Response to DMPQ IR	/0/39, 40
Feb 14, 2018	Updated FDA Form 356h	/0/41

2. Background

Human babesiosis is a tick-borne zoonotic disease caused by infections of humans with intra-erythrocytic protozoa of the genus *Babesia*. Babesiosis can also be transmitted by transfusion of blood and blood products and by solid organ transplantation collected from an infected donor. Human babesiosis is transmitted in many parts of the world, but the highest prevalence is reported in the United States. The first documented human case of babesiosis in the U.S. was reported in 1968. The majority of the U.S. babesiosis cases are caused by *B. microti*, the species that is prevalent in the Northeast and upper Midwest. A few other *Babesia* species such as *B. duncani* and related

organisms are implicated in transmission of *Babesia* in several western U.S. states, while "*B. divergens*-like" agents have been reported in multiple U.S. states.

A vast majority of *B. microti* infections are asymptomatic and never diagnosed. While the precise duration of *B. microti* infections in healthy adults is not known, in limited studies, the parasitemic period is reported to last from 2 to 7 months, but may also persist for more than two years. Although *Babesia* transmission is seasonal and coincides with tick activity (traditionally May-September), both tick-borne and transfusion-transmitted infections resulting from chronic unresolved parasitemia are reported year-round. The proportion of *Babesia* infections that persist as asymptomatic, chronic infections is not known. Asymptomatic individuals are difficult to recognize and, therefore, transfusion of blood and blood components collected from them may result in transfusion-transmitted babesiosis (TTB), leading to a potentially fatal clinical outcome in elderly or immunocompromised blood component recipients.

The Imugen *B. microti* NAT is an (b) (4) blood screening test intended for the detection of specific *B. microti* DNA. The NAT can be used as a standalone blood screening assay to provide testing of blood donations for evidence of *B. microti* infection. The clinical and analytical studies to support this intended use were conducted under the IND 14532 and its related amendments. The testing using the investigational *B. microti* NAT was performed within Imugen's clinical laboratory by trained staff using dedicated, qualified equipment and instrumentation in assigned, dedicated areas.

Multiple pre-submission discussions on the regulatory pathway were conducted with FDA under IND 14532/24, 26, 27 (June 21, 2013, August 02, 2013, September 23, 2013, October 25, 2013, response on February 7, 2014). A type B meeting request was received on April 4, 2014 and the face to face meeting was held on June 9, 2014. The sponsor proposed to submit a single BLA for NAT and Arrayed Fluorescence Immunoassay (AFIA) assays as a combined system. FDA maintained that two separate BLAs were needed; as each device contains a unique licensable component. Two BLAs were submitted on May 12, 2015.

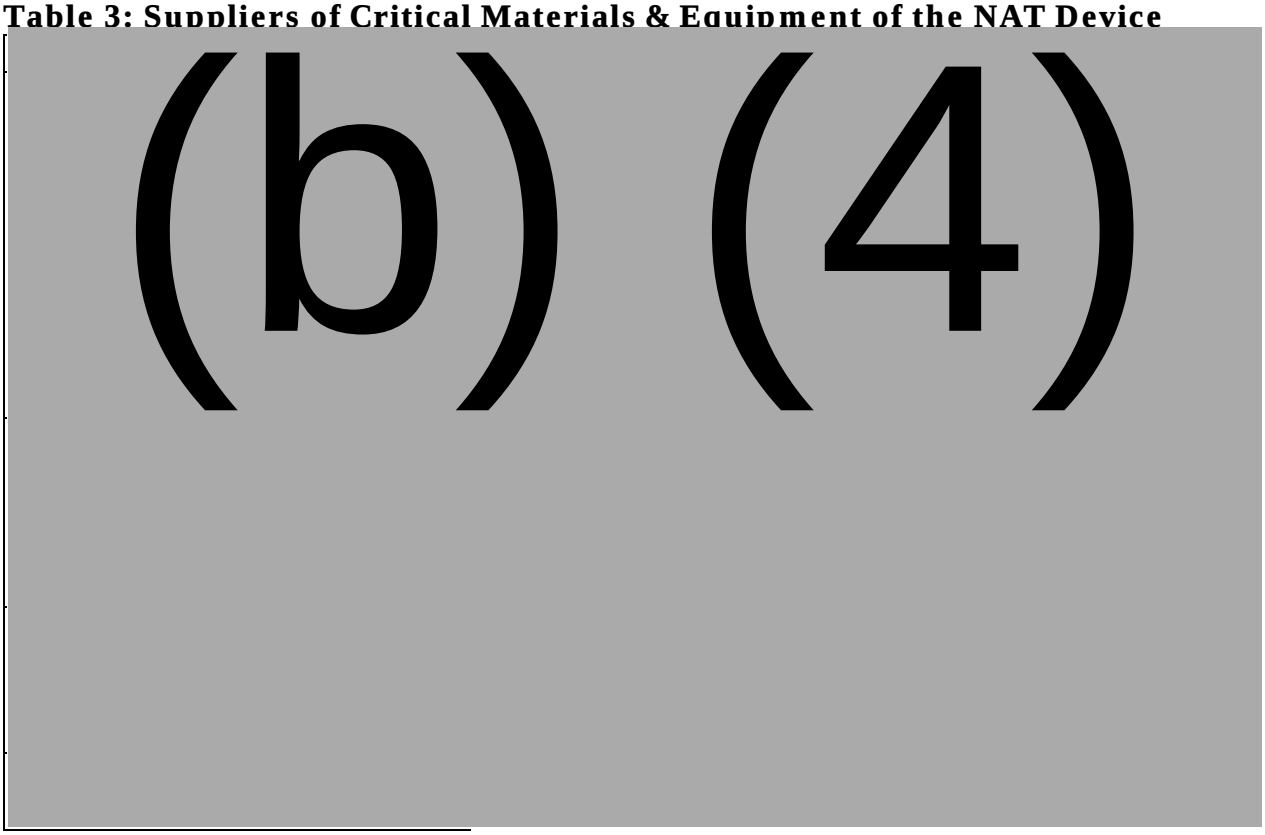
The *B. microti* NAT is based on (b) (4) and utilizes the (b) (4) automated nucleic acid purification instrument in combination with the (b) (4) qualitative detection of *B. microti* DNA in EDTA anti-coagulated whole blood samples. The assay employs an internal control of endogenous human RNA (to confirm amplification of a known RNA source in human samples) and *B. microti*-specific assay controls (high positive, low positive, and negative). To monitor the contamination during the process, no template controls (b) (4) are run with every assay run. Custom software called (b) (4) is used to collect and report data for blood donor sample testing within the Imugen facility.

3. Chemistry Manufacturing and Controls (CMC)

a) Manufacturing Summary

The *B. microti* NAT consists of *B. microti* specific primers and probes; human 18S sequence specific primers and probes; amplification reagents; and positive and negative controls. The materials and equipment required to perform the *B. microti* NAT are either purchased from suppliers or manufactured by Imugen. Table 3 summarizes the critical materials and equipment that are used in the manufacture of the NAT device along with the supplier used.

Table 3: Suppliers of Critical Materials & Equipment of the NAT Device



Custom (b) (4) *Babesia* 18S rRNA primers and probes, human 18S rRNA primers and probes, and (b) (4). The supplier provides release testing results on the lot-specific certificate of analysis that are reviewed as part of the Imugen's incoming acceptance process. The oligos are received in (b) (4) by Imugen with sterile water purchased from (b) (4). Each component is release tested with the Imugen *B. microti* NAT using positive and negative controls and must meet performance specifications. (b) (4) and the kits are tested with the *B. microti* NAT using positive and negative controls, and must meet performance specifications. Imugen receives *B. microti* infected (b) (4) blood from the (b) (4) as a contract service. The release testing is performed at Imugen and the (b) (4) blood must meet specifications for percent (b) (4) and for the absence of non-*Babesia* parasites and bacterial contamination.

Two of the critical reagents used in the *B. microti* NAT are manufactured at Imugen. Table 4 lists the components manufactured by Imugen.

Table 4: NAT Device Components Manufactured by Imugen

Component	Description
Positive NAT control (high positive and low positive)	The positive controls consist of characterized *Babesia* infected (b) (4) whole blood diluted in *Babesia* negative human whole blood to appropriate concentration levels. The controls are aliquoted into vials and release tested per SOP LAB-MFG-10. Positive controls are used to confirm the detection of *B. microti* nucleic acid at high and low levels with *B. microti* NAT, and to confirm the extraction step.
Negative NAT control	The negative NAT control consists of human whole blood characterized as *Babesia* negative. The controls are aliquoted into vials and release tested per SOP LAB-MFG-12. The negative control is used to ensure that there are no interferences in the NAT testing, and to confirm the extraction step.

The critical components of the NAT assay; *B. microti* specific primers and probes, human 18S sequence specific primers and probes, (b) (4) and positive and negative controls are final release tested and approved in combination to be used in the *B. microti* NAT device. The Finished Device Lot must meet release specifications prior to release, for use in blood donor screening at Imugen as per SOP LAB-AQC-MOL-102.

Review Issues: During the review, the following major issue was raised and resolved:

i. Low positive control near (b) (4) LOD: The low positive control used in the assay was around (b) (4) LOD and would fail to detect any assay deterioration trend (if it happens). The issue was communicated to the sponsor in the CR letter dated September 29, 2015. The sponsor switched the low positive control to (b) (4) LOD in March 2016 and the data was submitted in response to the CR letter on December 14, 2016 and the issue was resolved.

b) CBER Lot Release

The lot release protocol template was submitted to CBER for review and found to be acceptable after revisions. A lot release testing plan was developed by CBER and will be used for routine lot release.

c) Facilities review/inspection

Facility information and data provided in the BLA were reviewed by CBER and found to be sufficient and acceptable. The facility involved in the manufacture of the *Babesia microti* Nucleic Acid Test (NAT) and blood donor screening for the presence of *Babesia microti* using the NAT test is Oxford Immunotec Inc. doing business as (d/b/a) Imugen.

The activities performed and inspectional history are noted in the table 5 below and are further described in the paragraphs that follow**.**

Table 5: Manufacturing Facilities for Imugen *Babesia microti* Nucleic Acid Test

Name/Address	**FEI number**	**Inspection/ Waiver**	**Justification /Results**
NAT Assay Manufacture & Blood Donor Screening IMUGEN, Inc.* 315 Norwood Park South Norwood, MA 02062	3003505473	Pre-License Inspection	DMPQ/OBRR/ORA March 6 – 10, 2017 VAI

*At the time of inspection, the company was known as IMUGEN, Inc. Subsequent to the inspection the name of the company was changed to Oxford Immunotec Inc. d/b/a Imugen.

A pre-license inspection of IMUGEN, Inc. was conducted from March 6 – 10, 2017, and at the end of the inspection, a Form FDA 483 was issued. The firm responded to the observations and the corrective actions were reviewed and found to be adequate. All inspectional issues were resolved and the inspection was classified as voluntary action indicated (VAI).

d) Environmental Assessment

The BLA included a request for categorical exclusion from an Environmental Assessment under 21 CFR 25.31(c). The FDA concluded that this request is justified as the manufacturing of this product will not alter significantly the concentration and distribution of naturally occurring substances and no extraordinary circumstances exist that would require an environmental assessment.

e) Container Closure

N/A

4. Software and Instrumentation

The following is a summary overview of software, instrumentation and risk management information provided to support a reasonable assurance that the device is safe and effective for its intended uses and conditions of use:

Versioning: Software: (b) (4) Build 1.0.5.5 (not for commercial release). Hardware: (b) (4) workstations in client/server configuration for processing, PCR testing, and reporting; all running on supported versions of Windows (Windows (b) (4) and Windows Server (b) (4)

Device Description: The system supporting *B. microti* NAT is comprised of an RNA/DNA extraction system, real time PCR system, and custom (b) (4) software. Custom software called (b) (4) is used to collect and report data for blood donor sample testing within the Imugen facility. It does not control laboratory equipment, but facilitates collection of data; stores batch, sample data, and test results where the data are acquired through barcode scanning, touch-screen and keyboard entry, and electronic file import. Final sample results are electronically transmitted via email or FTP to the submitting entity.

Risk Management: The final risk assessment included 2 Excel spreadsheets with a total of 185 risks fully characterized with explicit hazards. The assessment of the risks identified included the following considerations; relevance to the software or product, cause, sequence of events, outcome, hazardous situation, premitigation and postmitigation assessment of risk, control measures, and the type of mitigation employed to reduce the risks to acceptable levels. The two risk documents address (b) (4) manufacturing and assay risks, and cybersecurity risks.

The risk analysis revealed 21 (b) (4) manufacturing and assay risks and 43 cybersecurity risks with a premitigation assessment of "Not Acceptable" related to alteration or deletion of stored data (including results), and reporting incorrect negative results. These were caused by issues with system access, performance, results reporting, interface and audit functionality. Primary hazardous situations included: 1) release of an infected unit for use in transfusion, 2) a unit inappropriately discarded, and 3) a unit delayed prior to release for transfusion reducing the donor blood supply. All risks have been reduced to "acceptable" or to "as far as possible" through multiple mitigations as per the applicant's risk management procedure. The applicant provided a further Risk/Benefit analysis to support that the overall residual risk is acceptable for exclusive in-house use of the assay. Overall, the applicant has established processes which should allow them to ensure that existing risks remain controlled, and that new risks can be easily assessed and mitigated.

Unresolved Anomalies: One unresolved anomaly related to incorrect highlighting of cycle threshold (Ct) values and possible false negative results was corrected during the course of this review, leaving no unresolved anomalies.

Testing: Verification and validation testing was performed in two parts, starting with initial Installation Qualification (IQ), Performance Qualification (PQ), and Operational Qualification (OQ) testing of the (b) (4) software. This was supplemented with additional testing identified by the newly-developed risk analyses to ensure that risk control measures associated with interoperability, performance and cybersecurity risks were correctly implemented.

Development Management: The software development activities included establishing detailed software requirements, linking requirements with associate verification tests, verification and validation testing, defect tracking, configuration management and maintenance activities to ensure the software conforms to user needs and intended uses.

Review Issues: During the review of this section, the following issues were raised and resolved:

i. The design control documentation originally provided was not developed under an adequate quality system. Through extensive interactions with the applicant, all software and instrumentation design control documentations were updated, and several processes supporting the quality system were revised or created. The most notable changes focus on the risk management processes and documentation and certain testing associated with previously-unidentified risks.

   a. Risk processes and associated artifacts were significantly updated and refined for better alignment with ISO 14971 "Medical devices – application of risk management to medical devices" and harmonized between the NAT and AFIA assays and submissions. The initial hazard analysis included 12 incompletely-developed risks. Use of the new process allowed the applicant to capture significantly more risks and mitigations at a level of detail appropriate to ensure that proposed risk control measures could be appropriately verified. Reanalysis of risk across the system led to several new and changed requirements and specifications, and the development of corresponding testing.

   b. Testing was initially limited to Installation Qualification (IQ), Performance Qualification (PQ), and Operational Qualification (OQ) testing of the (b) (4) software. The black box testing (IQ/PQ/OQ) was used to assess performance of the completed system, but did not include all verification testing necessary to ensure that certain error checking works correctly, that individual software components meet their specifications and that the interface among components is comprehensive, complete and correct. In response to deficiencies, additional unit and integration testing was developed and performed. This focused on higher level risks associated with errors and unexpected conditions related to user inputs and workflow, file import errors from the (b) (4) instrument, database integrity and performance, and cybersecurity mitigations related to data loss or corruption, improper access and improper software patching.

   c. Additional cybersecurity mitigations include a significantly enhanced Information Technology Security Policy and a new Disaster Recovery Plan Policy to both protect and recover from disruptions from equipment or application failure, database corruption, human error or sabotage, hacking, malicious attacks and other hazards associated with critical operations.

ii. The applicant made the following changes to improve safety and effectiveness of the device and supporting IT infrastructure, as a result of the identified review issues:

a. A software anomaly that could allow incorrect highlighting of Ct values and possible false negative results was corrected, tested and resolved.

b. The database server was upgraded from Windows Server (b) (4) (currently beyond End of Service date) to Windows Server (b) (4)

c. Cybersecurity protections were added to the shared IT infrastructure environment where the assay is performed.

5. Analytical Studies

The sponsor performed non-clinical/analytical studies to investigate and describe the functionality of the *Babesia microti* Nucleic Acid Test under certain conditions.

Sample requirements, storage and stability

The *B. microti* NAT uses EDTA whole blood. Specimens may be stored at Imugen in the refrigerator, 2-8°C, until testing is performed (within 48 hours). The sponsor assessed the stability of the whole blood samples for the *B. microti* NAT to assign shelf-life at various storage temperatures (b) (4) , 5±3°C, (b) (4) for sample storage, shipping, and handling. A total of 16 EDTA whole blood samples (8 *B. microti* NAT negatives and 8 *B. microti* NAT positives) were stored at the designated temperatures and tested at periodic intervals. The data showed that both the *Babesia* positive and negative samples were stable for at least (b) (4) The shipping instructions in Table 6 are provided to the blood centers for shipping the blood samples to Imugen.

Table 6: Shipping Conditions of Blood Specimens for Blood Donor Screening by NAT

Assay	Sample type	Storage /transport conditions
***B. microti* NAT Samples**	**Whole Blood:** EDTA (lavender top blood draw tube). 5 mL of whole blood is requested for testing. A minimum volume of 1 mL may be submitted.	Whole blood may be transported at ambient temperature within 48 hours of collection.

IVD / Kit stability

A stability study was performed to define a stability claim of each Finished Device Lot of the *B. microti* NAT system. This includes control components: 1) High Positive Control Eluate (*B. microti* DNA), 2) Low Positive Control Whole Blood, and 3) Negative Control Whole Blood; and the master mix components: a) (b) (4) b) *B. microti* Sequence Detection Primers; c) (b) (4) *Babesia* Probe; d) Human 18S Sequence Detection Primers; and e) (b) (4) 18S Probe. Three Lots were manufactured and were release tested per the SOPs. The finished device stability was assessed at different time points by the standard release test (Table 7).

Table 7: Stability Testing Timepoints

	T=0	3 Month	6 Month	9 Month	(b) (4)
Lot 1	Pass	Pass	Pass	Pass	
Lot 2	Pass	Pass	Pass	Pass	
Lot 3	Pass	Pass	Pass	Pass	

The claimed shelf life for individual assay components is 9 months. The expiration is determined by the earliest expiration date of a component within the finished device lot.

Limit of detection/Repeatability

Since no *B. microti* International Standard is available, the sponsor used a well characterized human clinical *B. microti* NAT positive specimen (b) (4) total *Babesia* parasites/mL) to determine the assay's limit of detection. The NAT positive specimen was diluted into *B. microti* NAT negative human whole blood to final concentrations of (b) (4) *Babesia* parasites/mL. Each concentration preparation was extracted in (b) (4) replicates using the (b) (4), and each (b) (4) Empirical data, presented in Table 8, indicate a (b) (4) detection limit for the NAT of approximately (b) (4) *Babesia* parasites/mL. The actual (b) (4) detection limit was extrapolated from the data. The results (% Detection vs. *Babesia* parasites/mL concentration) were plotted and the (b) (4) (b) (4) detection frequency limits were extrapolated from the graph. The (b) (4) detection frequency limit for the *B. microti* NAT was determined to be (b) (4) *Babesia* parasites/mL as extrapolated from the graph. The (b) (4) detection frequency were determined to be (b) (4) *Babesia* parasites/mL and (b) (4) *Babesia* parasites/mL respectively.

Table 8: *B. microti* NAT Limit of Detection

(b) (4)

Endogenous interferences

The sponsor assessed analytical specificity of the *Babesia microti* Nucleic Acid Test with the following endogenous substances: Elevated total proteins (20), Elevated Bilirubin (20), Lipemic (20), Elevated Triglycerides (20), Alkaline Phosphatase (20), Anti-

Nuclear Antibodies (ANA) (20), and Rheumatoid Antibody (RA) (7). The samples were spiked with (b) (4) LOD of *B. micro*ti target and were tested by the *B. microti* NAT with spiked and unspiked specimens. No interference was demonstrated in unspiked samples. There were no false negative results observed in the samples spiked with *B. microti*.

Cross-reactivity

The assay specificity of the *Babesia microti* Nucleic Acid Test is based on the specificity of the primers and the probes. The assay specificity was evaluated by testing cross-reactivity against the organisms listed in Table 9 below.

Table 9: Potential NAT Cross-reactive Organism

Organism	ATCC Strain	Target Concentration
Hemophilus influenza	(b) (4)	
Escherichia coli		
Pseudomonas aeruginosa		
Candida albicans		
Staphylococcus aureus		
Streptococcus pyogenes (Group A)		
Streptococcus pneumoniae (pneumococcus)		
Streptococcus faecalis (Enterococcus)		
Borrelia burgdorferi		
Plasmodium falciparum		

(b) (4) *Babesia* negative whole blood samples were spiked with (b) (4) cultured microorganisms listed above at (b) (4). The samples were spiked with (b) (4) LOD of *B. microti* target and were tested by *B. microti* NAT with spiked and unspiked specimens. For *Plasmodium falciparum*, 20 parasite positive clinical samples were combined (b) (4) with *B. microti* negative or positive (b) (4) LOD) packed red blood cells to assess potential cross-reactivity. The samples spiked with *B. microti* had reactive results and all the unspiked samples had negative results for *B. microti*, with valid Ct values of (b) (4) for the human 18S internal control specific primers and probe. These results demonstrated that these organisms did not interfere or cross react with the *B. microti* NAT.

Cross-contamination

Cross-contamination/carryover from (b) (4) was evaluated on the (b) (4) by testing multiple extraction runs of (b) (4) test specimens, alternating high positive and negative controls in adjacent wells and rows, on (b) (4) extraction runs. This tested for possible well-to-well contamination and for possible run-to-run contamination. (b) (4) Additionally, a study was run in which a positive control was prepared at a higher concentration of (b) (4) *Babesia* parasites/mL, and the study was repeated on a (b) (4). This run

checked for possible cross-contamination at very high concentrations of *Babesia*. A (b) (4) of *Babesia*-negative controls was run to check for possible run-to-run contamination. There was no cross-contamination/carryover observed from well-to-well, row-to-row, or run-to-run in the (b) (4)

Precision study

The precision study for the *Babesia microti* Nucleic Acid Test was performed under protocol DOC PRO-26 in IND #14532. Precision was assessed across the following factors: (b) (4) Testing is summarized in Table 10.

Table 10: Expected Results and Total # for NAT Precision Studies

(b) (4)

The results showed 100% agreement with expected results for all samples and controls (Table 11).

Table 11: Summary of Overall Results

(b) (4)

(b) (4)					

[1] External controls were included on each day that testing was performed.

Review issues: During the review of this section, the following issues were raised and resolved:

i. Endogenous interference and cross-reactivity: During the review of endogenous interference and cross-reactivity studies it was observed that studies did not evaluate assay performance with some key potentially interfering agents and cross-reactive organisms. Additionally, the *B. microti* parasite stock used to spike the test samples was not well characterized, leading to unexpected results in assay controls. The deficiencies were communicated in the CR letter of September 29, 2015. The sponsor updated the protocol and the study was repeated with well characterized *B. microti* parasite stock. The data were submitted in response to the CR letter on December 14, 2016 and the issues were resolved.

ii. Precision studies: The precision studies failed to capture intra- and inter-assay variability, intra- and inter-lot variability, inter-operator variability and inter-instrument variability and the studies were updated in response to the CR letter of December 14, 2016. The results of the NAT precision study were analyzed in two ways, qualitatively (agreement) and quantitatively (Ct Value). The statistical reviewer recommended removal of the variability analysis conducted on the Ct values since the estimated variability for Ct values was not accurate. It was requested that the results should be reported as percent agreement. The issue was communicated in the CR letter of June 13, 2017. The sponsor responded to the CR letter on October 10, 2017 and the issue was resolved.

6. Clinical Studies

a) Clinical Program

The clinical studies supporting this application were performed under IND #14532. Blood donors (prospective and repository) from the American Red Cross (ARC), Memorial Blood Centers of Minnesota (MBC), and the Rhode Island Blood Center (RIBC) were screened under the IND protocols for evidence of *B. microti*. Blood donors in regions predicted to be highly endemic, low-medium endemic, and non-endemic for *B. microti* were included in the clinical study to evaluate the performance of the Imugen *B. microti* NAT.

Clinical specificity

The clinical specificity study #2 was designed to screen 13,192 archived blood samples collected by American Red Cross for *B. microti* by the Imugen *B. microti* NAT. The samples were collected from high endemic regions of Connecticut (CT) and Massachusetts (MA), low-mid endemic regions of Minnesota (MN) and Wisconsin (WI), and non-endemic regions of Arizona and Oklahoma. Donors who initially test NAT positive were retested in triplicate to confirm the positive finding prior to reporting a NAT positive result. An inconclusive result was

reported if the donor had an initial positive result and three negative findings in the confirmatory replicates.

The specificity of the Imugen *B. microti* NAT assay in non-endemic donors (AZ and OK) was found to be 100.00% {3969 / (3969 + 0) x 100%} with 95% CI of 99.91% to 100%. No NAT positive or inconclusive results were observed.

The specificity of the investigational NAT test in endemic donors (CT, MA, MN, and WI) was assessed by comparison to the arrayed fluorescence immunoassay (AFIA). Enhanced PCR (ePCR) was utilized as a test to characterize NAT inconclusive as well as AFIA positive/NAT negative index samples. In contrast to Imugen's conventional NAT protocol whereby a single (b) (4) aliquot of the extracted DNA is amplified, the ePCR technique amplifies (b) (4) aliquots (typically (b) (4) replicate amplifications per sample) of the total extracted DNA (or until the entire extraction volume is extinguished). This enhances the sensitivity of the assay by increasing the probability of detecting any *Babesia*-specific DNA present in low concentration in the entire extracted sample volume. There were 7 NAT positive and 1 NAT inconclusive donor specimens identified out of 9,223 specimens tested and collected from the endemic regions. All 7 NAT positive samples in this study were also AFIA positive. The NAT inconclusive donor index sample was negative by AFIA, ePCR, and Western Blot (WB) (IgM and IgG). The follow-up sample drawn 12 months after the index donation testing was negative by NAT, AFIA, ePCR, and WB (IgM and IgG). This donor revealed no evidence of exposure to *Babesia*, and the initial non-reproducible NAT result is interpreted as a false positive. The 24 AFIA positive/NAT negative donors were ePCR negative; and represent seroconverted donors who have either cleared *B. microti* parasites or the parasitemia is below the assay's limit of detection. The specificity of the NAT assay in donors from an endemic area (retrospective study) was found to be 99.99% {9189 / (9189 + 1) x 100%} with 95% CI of 99.94% - 100.00% (Table 12).

Table 12: Comparison of NAT to AFIA[i] in Endemic Region

	AFIA Positive	AFIA Inconclusive	AFIA Negative	Total
NAT Positive	7	0	0	7
NAT Inconclusive	0	0	1[ii]	1
NAT Negative	24[iii]	2	9189	9215
Total	31	2	9190	9223

i. AFIA ≥128 Cutoff
ii. NAT inconclusive result was initially reactive sample that was negative upon re-test.
iii. AFIA positive/NAT negative samples were ePCR negative.

The prospective study (Study 3a) was intended to test the specificity of the investigational tests in prospectively collected blood donor samples in *B. microti* endemic areas. The areas included were Connecticut, Massachusetts, Minnesota and Wisconsin. A total of 88,904 linked whole blood donors were screened; and

62 (0.07%) NAT positive donors and 1 (0.001%) NAT inconclusive donor were identified in the study. The 1 NAT inconclusive donor was AFIA positive, WB positive and ePCR positive at index. A follow-up sample at 27 days after index donation testing was NAT negative, ePCR positive, and antibody positive by AFIA (1:512) and WB (IgG). The donor had a 1:64 AFIA titer on the day 551 post index sample; and the titer fell below 1:64 at day 634. Nine (0.01%) window period donors were identified in the study, three from Massachusetts, five from Connecticut and one from Minnesota. Out of the 9 cases, 8 donors had at least one follow-up and 7 of them seroconverted (AFIA and WB). The 275 AFIA positive/NAT negative donors were ePCR negative; and represent seroconverted donors who have either cleared *B. microti* parasites or the parasitemia is below the assay's limit of detection.

The specificity of the NAT assay was established by comparison to the AFIA assay. Of the 88,904 donor samples tested, one donor whose index sample was NAT positive and AFIA negative, with no follow up sample available, was excluded from the specificity calculation. The specificity of the NAT assay in endemic donors (prospective study) was found to be 99.99% {[88564 / (88564 + 1)] x 100%} with 95% CI of 99.99% to 100 % (Table 13).

Table 13: NAT Specificity against AFIA

	AFIA Positive	AFIA Inconclusive	AFIA Negative	Total
NAT Positive	60[i]	0	1[ii]	61
NAT Inconclusive	1	0	0	1
NAT Negative	275[iii]	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. AFIA positive/NAT negative samples were ePCR negative.

Under the prospective study (Study 3b) testing of the blood donors was performed in Minnesota (a geographical area categorized as a low endemic area). A total of 1,187 whole blood donors were screened for *B. microti*. There were no positive or inconclusive findings identified in this study. No cases of transfusion transmitted babesiosis were reported or documented from any screened units of blood in this study.

Clinical sensitivity

The evaluation of the sensitivity of the *Babesia microti* Nucleic Acid Test was performed using 72 confirmed *B. microti* blood film positive clinical samples. To avoid bias, 23 *B. microti* negative samples were included in the testing (24% of study samples). Operators were blinded to the infectious status of all specimens prior to testing. The *B. microti* NAT detected 100% of the positive samples with 95% CI of 95.01% to 100.00%. Twenty-two of the 23 negative specimens were negative by NAT, one was inconclusive. The inconclusive result had a high Ct value; it tested negative upon repeat testing.

Review Issues: During the review of this section, the following major issue was raised and resolved:

i. Sample selection criterion for clinical sensitivity: The sponsor used the investigational test for sample selection to perform the clinical sensitivity study. The issue was communicated in the CR letter of September 29, 2015. The sponsor repeated the study using an updated criterion for samples to be blood film positive for *B. microti* and the data were submitted in response to the CR letter on December 14, 2016 and the issue was resolved.

Label considerations
N/A

Bioresearch Monitoring

Bioresearch Monitoring (BIMO) inspections were conducted at one clinical site and one sponsor site that participated in the conduct of Study BNATIFA-10. The inspections did not reveal any issues that impact the data submitted in this application.

b) Pediatrics
N/A

c) Other Special Populations
N/A

7. Advisory Committee Meeting

N/A

8. Other Relevant Regulatory Issues

N/A

9. Labeling

The Advertising and Promotional Labeling Branch (APLB) found the proposed Standard Operating Procedures (SOPs) to be acceptable from a promotional and comprehension perspective.

10. Recommendations and Risk/ Benefit Assessment

a) Recommended Regulatory Action
The Review Committee reviewed the original submission and related amendments; conducted a pre-license inspection, reviewed the sponsor's response to 483 observations. All review issues have been resolved; therefore,

the Review Committee recommends licensure of the Imugen *Babesia microti* Nucleic Acid Test.

b) Risk/ Benefit Assessment

The Imugen *Babesia microti* Nucleic Acid Test is the first assay intended for detection of *B. microti* DNA in blood donations. The assay has an estimated 95% LOD of (b) (4) *Babesia* parasites/mL. The clinical studies demonstrated a sensitivity of 100% (95% CI of 95.01% - 100.00%), indicating low probability of a false negative result. Among ~90,000 units of blood tested from endemic areas with the *Babesia* NAT, no cases of transfusion transmitted babesiosis have occurred. The assay specificity of 99.99% (95% CI of 99.99-100%) in clinical trials suggests the low probability of false positives. Adverse events that may occur would be a false negative test result that would permit *Babesia* infected blood to be transfused; or false positive result that would result in discarding healthy, usable blood and loss of the donor who would be deferred. The Imugen *Babesia* NAT with high clinical sensitivity and specificity will significantly improve blood safety and public health by reducing the transfusion of *B. microti* infected blood, which can be fatal in susceptible recipients.

c) Recommendation for Postmarketing Activities

No postmarketing activities have been proposed for this application.