Summary Basis of Regulatory Action

Date: March 06, 2018

From: Robert Duncan, Chair of the Review Committee

BLA/ STN#: 125589

Applicant Name: Oxford Immunotec Ltd.

Date of Submission: May 12, 2015

Complete Response Letter 1: September 29, 2015

Resubmission 1: December 13, 2016

Complete Response Letter 2: June 13, 2017

Resubmission 2: October 10, 2017

MDUFA Goal Date: April 11, 2017

Proprietary Name: Imugen *Babesia microti* Arrayed Fluorescence Immunoassay (AFIA)

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

Intended Use/Indications for Use:

The Imugen *Babesia microti* Arrayed Fluorescence Immunoassay (AFIA) is intended for qualitative detection of antibodies to *Babesia microti* in human plasma (EDTA anti-coagulated) samples.

This test is intended for use as a donor screening test to detect antibodies to *Babesia microti* in plasma 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.

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

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

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

Review Office Signatory Authority: Nicole Verdun, MD, 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*	 Luisa Gregori Pawan Jain Rana Nagarkatti Rana Nagarkatti	 Jan 8, 2018 Apr 18, 2017 Jun 1, 2017 Jun 1, 2017
Statistical Review *Clinical* *Non-Clinical*	Paul Hshieh	Dec 29, 2017
CMC Review *CMC (DETTD)* *Facilities Review (OCBQ/DMPQ)* *Establishment Inspection Report (OCBQ/DMPQ)*	 Ranadhir Dey Sreenivas Gannavaram Alain Debrabant Lori Peters Lori Peters Sean Byrd Justine Corson (ORA) Robert Duncan (DETTD) Babita Mahajan (DETTD)	 Jun 1, 2017 Feb 15, 2018 Dec 15, 2017 Feb 12, 2018 Feb 12, 2018
Labeling Review *APLB (OCBQ/APLB)* *Product Office*	 Dana Jones Robert Duncan	 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 8, 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* Arrayed Fluorescence Immunoassay (AFIA). This is the first serology 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* AFIA 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 607593). 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 125589 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 received on December 14, 2016 and the amendment was classified as a Class 2 resubmission. An Information Request (IR) Letter was sent on February 24, 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
BL125588: Imugen *Babesia microti* Nucleic Acid Test (NAT) – Received May 12, 2015

Table 2: Chronological Summary of Submission and FDA Correspondence

Date	Action	Amendment to BL125589
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 29, 2015	Complete Response Letter	
Jul 1, 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	/0/9
Dec 13, 2016	Response to CR Letter; Resets the goal date to Jun 14, 2017	/0/12
Feb 24, 2017	FDA IR on CR responses	
Mar 6-10, 2017	Pre-license inspection; FORM FDA 483 issued	
Mar 17, 2017	Response to IR letter	/0/13
Mar 17, 2017	Change in BLA ownership filed	/0/14

Apr 5, 2017	FDA IR on Lot release template	
Apr 13, 2017	Request for face-to-face meeting on manufacturing scale up (BQ170100)	/0/16
Apr 14, 2017	FDA IR on software	
Apr 17, 2017	483 Response	/0/15
Apr 20, 2017	Teleconference to discuss software issues	
May 3, 2017	Sponsor meeting packet	/0/18
May 5, 2017	Sponsor submission of Lot Release Template	/0/17
May 10, 2017	Face to Face Meeting to discuss manufacturing scale-up	BQ170100
May 18, 2017	Call request to discuss software issues	
May 18, 2017	Sponsor changes point of contact	/0/21
May 23, 2017	Sponsor response to IR (software)	/0/22
Jun 5, 2017	483 Resolution Progress	/0/23
Jun 13, 2017	483 Resolution Progress	/0/24
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	/0/27
Oct 26, 2017	Updated Lot release template	/0/28
Nov 9, 2017	FDA IR for software issues	
Nov 20, 2017	Response to software IR	/0/29
Nov 30, 2017	FDA IR for software (AFIA Risk Assessment)	
Dec 1, 2017	Interactive review of performance data, Lot Release testing	/0/30, 31
Dec 4, 2017	Response to software IR	/0/32
Dec 5, 2017	FDA IR for software	
Dec 15, 2017	Information request – DMPQ 483 issues	
Dec 18, 2017	Teleconference (DMPQ)	
Dec 19, 2017	Response to software IR	/0/33
Jan 9, 2018	Response to DMPQ IR	/0/34
Feb 2, 2018	FDA IR for DMPQ 483 issues	
Feb 6, 2018	Response to DMPQ IR	/0/35
Feb 14, 2018	Updated SOP and FDA Form 356h	/0/36, 37

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. 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 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 the other "*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 around. 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* AFIA is an *in vitro* blood screening test intended for the detection of specific antibodies to *B. microti* in donor plasma. The AFIA 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* AFIA 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 AFIA and Nucleic Acid Test (NAT) 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* AFIA is based upon a conventional indirect immunofluorescent assay (IFA) and is used for detecting the presence of specific antibodies to *B. microti* in EDTA anti-coagulated plasma specimens. The test employs *B. microti* infected (b) (4) erythrocytes, as an antigen source, fixed to glass slide wells and a (b) (4) F(ab')2 anti-human IgG H+L chain specific, (b) (4) conjugated antibody as a detector of bound human *B. microti*-specific antibody. The fluorescence is detected in the wells of the slide employing a microscope equipped with a fluorescence illumination system. Positive and negative control plasma is employed on each slide. The positive control is expected to produce a visible fluorescence pattern, while the fluorescence pattern should not be observed with the negative control plasma. 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 manufacturing process for in vitro substance begins with preparation of *B. microti*-infected (b) (4) red blood cells (RBC) that will function as antigen in the AFIA.

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

Supplier	Materials and equipment
(b) (4)	(b) (4), glass slides
(b) (4)	*B. microti* infected (b) (4) whole blood
(b) (4).	F(ab')2 fragment of (b) (4) IgG (specific for human H and L IgG chains) labeled with (b) (4) catalog number (b) (4)
Prepared at Imugen using (b) (4) plasma from an individual, previously infected with *B. microti*, aliquoted, (b) (4) and release tested by (b) (4) SOP LAB-MFG-5	Positive Control
Prepared at Imugen from (b) (4) plasma from a non-infected individual, aliquoted, (b) (4) and release tested by (b) (4) SOP LAB-MFG-5	Negative Control
(b) (4)	(b) (4) models of fluorescent microscopes

The in vitro product is manufactured and assembled from commercially available components entirely at the Imugen facility at 315 Norwood Park South, Norwood, MA 02062.

The first component is manufactured by (b) (4) glass slides that are purchased with (b) (4) wells on their surface, fixing the red cells to the slides and storing at (b) (4) according to SOP LAB-MFG-14&15. In process testing of the slide preparation includes:

(b) (4)

The Finished Device is composed of a lot of RBC coated slides, a lot of (b) (4) IgG (specific for human H and L IgG chains) labeled with (b) (4) a lot of assay negative control and a lot of assay positive controls.

Final release testing of the Finished Device Lot involves testing samples of each component lot from the (b) (4) of production runs together in AFIAs with a QC panel as the specimens to be tested. Each QC panel member has pre-specified acceptance criteria following SOP LAB-AQC-SER-558.

Table 4: Finished Device Lot Release Testing Specifications

Control/QC Panel Sample Number[1]	AFIA Target Titer	Titer Range Specification
AFIA Negative Control, (b) (4) dilution	(b) (4)	
AFIA Positive Control, (b) (4) dilution	(b) (4)	
(b) (4)		

[1]Controls and QC panel members prepared at Imugen from (b) (4)

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

i. Thorough genetic characterization of the *B. microti* source: The sponsor did not provide sufficient information to establish the identity of the *B. microti* parasites used to establish the master seed stock. In response to the FDA's CR letter of September 29, 2015, the sponsor provided genetic typing by (b) (4) to adequately characterize the stock.

ii. Establishment of a master and working cell bank: The sponsor did not provide sufficient documentation of a system to ensure consistent quality of the *B. microti* source. In response to the FDA's CR letter of September 29, 2015, the sponsor established a program, described in LAB-MFG-29, of a master cell bank and working cell banks that is acceptable.

iii. (b) (4) protocols: The injection of the *B. microti* source material into (b) (4) maintenance of infected (b) (4) and collection of blood at the proper time are all performed at the (b) (4). The sponsor did not present any formal arrangement with (b) (4). for this service which is essential for the consistent manufacturing of the AFIA product. FDA required that a contract agreement covering all the essential steps in the process of producing infected (b) (4) blood be covered by a signed agreement in the CR

letter of September 29, 2015. The sponsor obtained an agreement that was reviewed by FDA and found acceptable.

iv. Finished Device Lot manufacturing: The sponsor presented a manufacturing and donor screening testing approach with each component remaining independent. In the CR letter of September 29, 2015, FDA required establishing a manufacturing practice that defines a Finished Device Lot of assay components, tests them together in a lot release test and requires assays to be performed with reagents from a single Finished Device Lot. In response to this CR letter, the sponsor described establishment of a Finish Device Lot system with appropriate release testing of the combined Finished Device Lot. Utilization of the Finished Device Lot system was observed at the pre-license inspection and found acceptable.

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 Arrayed Fluorescence Immunoassay (AFIA) and blood donor screening for the presence of *Babesia microti* using the AFIA 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* Arrayed Fluorescence Immunoassay

Name/Address	FEI Number	Inspection/ waiver	Justification /Results
AFIA 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 supported versions of Windows (Windows (b) (4) and Windows Server (b) (4)

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

Risk Management: The final risk assessment included 3 Excel spreadsheets with a total of 352 risks fully characterized. The assessment of the risks identified included the following considerations; explicit hazards, relevance to the software or product, cause, sequence of events, outcome, hazardous situation, premitigation and postmitigation assessment of risk, controls measures, and the type of mitigation employed to reduce the risks to acceptable levels. The three risk documents address (b) (4) manufacturing and assay risks, cybersecurity risks, and AFIA processing related risks.

The risk analysis revealed 18 (b) (4) manufacturing and assay risks, 58 AFIA processing-related 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. AFIA-processing causes include batch inhomogeneity and inconsistency, imprecise measurements, specimen identification errors, stability problems, problems preparing samples, use error, uncertainties with cutoffs and interfering factors, bio-contamination, incorrect formulation, degradation, inadequate labeling, inadequate instructions, inadequate hazard warnings, incompatibilities with consumables and other devices, and misrepresentation of results. Primary hazardous situations include: 1) release of an infected unit for use in transfusion, 2) a unit inappropriately discarded, and 3) a unit delayed prior to transfusion or discarded, reducing the donor blood pool. All risks have been reduced "as far as possible" though multiple mitigations, and the applicant has provided a further Risk/Benefit analysis to support that the overall residual risks are acceptable. 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: No unresolved anomalies have been reported.

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

b. 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* Arrayed Fluorescence Immunoassay under certain conditions.

Sample requirements and storage

The *B. microti* AFIA uses EDTA plasma derived from whole blood. Specimens may be stored at Imugen in the refrigerator, 2-8°C, until testing is performed within 48 hours.

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

Assay	Sample type	Storage /transport conditions
***B. microti* AFIA 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* AFIA system, including: 1) (b) (4) Conjugated (b) (4) Anti-human IgG Antibody; 2) High Positive Control; 3) Negative Control; and 4) *B. microti* Coated Slides, when stored at (b) (4) the appropriate and designated storage condition. (b) (4) Finished Device Lots were manufactured and release tested according to the SOPs and tested by the standard release test at three-month time points from time 0 to Month (b) (4) Test results have been provided through the (b) (4) month for all (b) (4) lots. Based on the current results, the expiration date of a Finished Device Lot of the AFIA is 6 months.

Table 7: Stability Testing Timepoints

Time Point	(b) (4)		
T=0	Pass	Pass	Pass
Month 3	Pass	Pass	Pass
Month 6	Pass	Pass	Pass

(b) (4)

Cutoff determination

(b) (4)

These combined results indicate a cutoff titer of 128 is sufficient for detecting exposure to *B. microti*.

Exogenous interferences

A study was performed with (b) (4) *B. microti* antibody negative human plasma samples and (b) (4) *B. microti* antibody positive (titer= 1:128) human plasma samples. Each sample was tested with no additive or with a spiked target concentration of (b) (4) of the following pathogen species: *Hemophilus influenza, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus, Streptococcus pyogenes* (Group A), *Streptococcus pneumoniae* (pneumococcus) and *Streptococcus faecalis* (Enterococcus).

All negative baseline and negative pathogen-spiked samples were negative at a 1:128 dilution. All positive baseline samples were spiked to a 1:128 dilution and all corresponding pathogen spiked samples maintained a 1:128 dilution. Thus none of the added pathogens interfered with the positive reaction nor caused reactivity where there was none.

Cross-reactivity

To test the *Babesia microti* AFIA for reactivity to potentially cross-reactive antibodies, clinical specimens from individuals infected with the following pathogens were obtained and run in the AFIA system at a dilution of 1:64 to identify potential false positives. Results were not titered to end point.

Table 9: Results of Testing Plasma Samples with Potentially Cross-reactive Antibodies

Antibody Specificity	Number of Samples	Total # AFIA Reactive[1]
Syphilis *(Treponema pallidum)*	30	0
Hepatitis C	30	0
Hepatitis B Surface antigen	30	1
Human Immunodeficiency Virus (HIV)	29	1
Chagas *(Trypanosoma cruzi)*	30	1
Lyme *(Borrelia burgdorferi)*	30	0
Malaria *(Plasmodium vivax)*	6	0
Malaria *(Plasmodium malariae)*	3	0
Malaria *(Plasmodium ovale)*	3	0
Malaria *(Plasmodium falciparum)*	24	4
Malaria (pooled *anti-Plasmodium)*	2	0
CMV (Cytomegalovirus)	60	3
EBV (Epstein-Barr Virus)	74	3
HSV-1 (Herpes Simplex I Virus)	73	3
HSV-2 (Herpes Simplex 2 Virus)	70	3
Rubella Virus	72	3
Toxoplasma *(Toxoplasma gondii)*	17	1
Schistosomaisis	10	1

[1] Positive results may be false positives caused by interference, or may be actual positives from people infected with Babesiosis.

No cross reactivity was observed for Lyme disease, a major co-endemic disease. Significant cross reactivity was not observed for any of the other pathogens except *Plasmodium falciparum*, a *Babesia* related intra-erythrocytic parasite. *P. falciparum* specimens (n=4) in the initial study were tested at a dilution of 1:64 (higher concentration than the final cutoff) and were obtained without documentation confirming the *P.f.* status or origin. Consequently, the cross reactivity study was extended by obtaining 20 specimens positive for *Plasmodium falciparum* antigens based on results of the (b) (4) Test for testing in the AFIA at a dilution of 1:128. None of the specimens were reactive with the *B. microti* AFIA and all the specimens tested *Babesia* reactive when they were spiked with high positive anti-*B. microti* antibodies. Therefore 4 of the 24 tested showed reactivity with the *B. microti* AFIA, which may react with anti-*Plasmodium* antibodies.

(b) (4) samples each that were reactive by testing for Syphilis, HCV, HIV, HBsAg, Chagas, Lyme, malaria or schistosomiasis were spiked with high positive anti-*B. microti* antibodies. These spiked positive samples all demonstrated no interference with the detection of anti-*B. microti* antibodies. None of these infectious diseases interfere with the *B. microti* AFIA.

Endogenous interferences

In this study, samples (b) (4) of each) with potentially interfering substances were spiked with high positive anti-*B. microti* antibodies to a target titer of 128.

Table 10: Results of Testing Endogenous Substances for Interference and Cross-reactivity

Interfering Substance (mean level)	Samples tested	# with unspiked reactive result[1]	# with spiked reactive result
Rheumatoid Antibody (1318u/mL)	19	0	5/5
Anti-Nuclear Antibody [ANA] (1:1344 titer)	40	3	25/25
Elevated Triglycerides/ Lipemic (2200 mg/dL)	27	1	10/10
Cholesterol/ Lipemic (355 mg/dL)	27	0	10/10
Alkaline Phosphatase (300U/mL)	19	1	5/5
Elevated Bilirubin/Icteric (26 mg/dL)	20	0	5/5
Elevated Total Protein (16 g/dL)	15	0	5/5

[1]Samples obtained were not pre-screened for exposure to *Babesia*, thus reactive results may be true positives.

Given the uncertainty of true prior *B. microti* infection in the ANA samples, 20 additional ANA plasma specimens were obtained and tested initially and after spiking with high positive anti-*B. microti* antibodies to a target titer of 128. None of the initial tests were reactive and all the spiked samples were reactive. Thus these results indicate that anti-nuclear antibodies do not cross-react nor interfere with the *B. microti AFIA*. None of the substances showed significant cross reactivity or interference with the *B. microti* AFIA.

Precision study

In Imugen's CR Letter Response received December 12, 2016 a Precision Study Report for the AFIA was included. It followed the guidelines in CLSI documents EP5-A3, EP12-A2 and EP5-A3. These guidelines were followed for within laboratory, (b) (4) operators and systems, 3 lots; (b) (4) testing schedule; Negative and positive controls included with each run; % Agreement, Score 95% CI for Repeatability, Between-Run (and Operator), Between-Day, Within-Laboratory.

The study utilized a panel of (b) (4)

(b) (4)

(b) (4)

(b) (4)

It is concluded that the Imugen *B. microti* AFIA performs with acceptable precision when challenged with near-cutoff level serum and plasma samples.

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

i. In the original submission, the sponsor reported data from pre-clinical and clinical studies with an AFIA result described as reactive when the sample was diluted 1:64 in some cases and only when the sample was reactive at a dilution of 1:128 in other cases. The FDA stated in a CR letter on Sep. 29, 2015 that consistent application of the assay cutoff that interprets reactivity at a dilution of the plasma sample equal to or greater than 1:128 as reactive, consistent with the assay cutoff study reported in the application, was required. The sponsor agreed and presented revised reports of the data with the 1:128 cutoff consistently applied.

ii. The only data on stability in the original submission was individual component stability studies. In a CR letter on Sep. 29, 2015, the FDA described the Finished Device Lot system that would be required and accompanying stability testing to establish shelf life with (b) (4) Finished Device Lots. The sponsor has Finish Device Lot stability testing results up to (b) (4) months and is planning to test up to (b) (4)

iii. The original submission showed 4 *Plasmodium* reactive samples that all reacted with the AFIA and 3 of 20 anti-ANA antibody plasma samples that reacted with the AFIA. This level of reactivity was not acceptable to the FDA; so additional testing was requested. Extended studies were performed to confirm the low level of cross-reactivity of *Plasmodium falciparum* infected samples and anti-ANA samples with the AFIA.

6. Clinical Studies

a) Clinical Program

The clinical studies supporting this application were performed under IND #14532. The study protocol under this IND was submitted on Oct. 13, 2010 to support the donor screening by both AFIA and NAT. Donor testing under IND is ongoing; however, the data collection for submission in the BLA was closed on Sept. 30, 2014.

Clinical specificity

The clinical specificity study was comprised of a retrospective study and a prospective study.

The retrospective study tested 13,192 asymptomatic blood donor specimens from the American Red Cross repository collected in 2010 and 2011 from six locations, two highly endemic areas (Connecticut and Massachusetts), two low-medium

endemic areas (Minnesota and Wisconsin) and two non-endemic areas (Arizona and Oklahoma). Blood donor samples consisted of whole blood that was separated into a whole blood fraction that was extracted for the NAT assay and a plasma fraction used for detection of anti-*B. microti* antibodies in the AFIA. All AFIA positive and inconclusive samples were tested with a research use only (RUO) Western immunoblot. All specimens were also tested by *B. microti* NAT as part of the IND studies for BLA 125588. Donors testing positive or inconclusive were deferred indefinitely and asked to submit additional blood samples for testing.

Specificity Calculations

In the non-endemic area, 3969 donor samples were tested. Among these, 3968 tested non-reactive with the *Babesia microti* AFIA. One specimen was AFIA reactive. Assuming the non-endemic specimens were all true negative for antibodies to *B. microti*, there were 3968 test negative /3969 true negative = 99.97% (95% CI = 99.86% - 100.00%) representing one measure of AFIA specificity.

As further analysis of the retrospective study, the reactive samples collected in the highly endemic and Low-medium endemic areas were retested with a RUO *Babesia* Western immunoblot to detect antibodies to *B. microti.* The Western immunoblot *B. microti* antibody assay was originally developed as a laboratory developed test and has been in use at Imugen for more than a decade. The antigen used for the Western Blot is *B. microti* (b) (4) The staining of specific bands on the blot is the criterion for *Babesia* reactivity.

Table 13: Comparison of AFIA to Western blot and NAT assays Among Endemic Region Donors

	NAT or WB positive	NAT or WB inconclusive	NAT and WB negative	Total
AFIA reactive	31	0	0	31
AFIA inconclusive	0	0	2*	2
AFIA non-reactive	11	1**	9178	9190

Total	42	1	9180	9223

*AFIA inconclusive results are defined as non-specific fluorescence upon re-test.
**NAT inconclusive results are initially reactive samples that show up negative upon re-test.

Specificity Calculations
9178 / (9178 + 2) x 100%= 99.98% (95% CI = 99.92% - 100.00%).

The prospective study was intended to test the specificity of the investigational tests in blood donor samples in *Babesia* endemic areas. The areas included were Connecticut, Massachusetts, Minnesota and Wisconsin.

Table 14: Prospective Study Results

	NAT and/or WB positive	**NAT and/or WB inconclusive**	**NAT and WB negative**	**Total**
AFIA reactive	291	4	33	328
AFIA inconclusive	0	1	2	3
AFIA non-reactive	9	0	88,564	88,573
Total	300	5	88,599	88,904

Specificity Calculations:
88,564/ (88,564+33+2) = 99.96% (95% CI = 99.94-99.97%).

Imugen provided a complete spreadsheet of the data collected in the retrospective and prospective studies. This data was analyzed by FDA statisticians compiling tables and calculating statistics independently from the ones provided by Imugen. No significant discrepancies were found in the data.

Clinical sensitivity
In the FDA Clinical Hold Letter dated December 10 2010, FDA requested that Imugen demonstrate the clinical sensitivity of this test in human samples that are blood-film positive for *B. microti*. Imugen performed a study to define the Clinical sensitivity of the *B. microti* AFIA assay for use in screening human donors of blood and blood components for transfusion. In this study, 72 blood-film confirmed *Babesia* infected samples were tested that allowed for the calculation of the confidence interval of the sensitivity estimate of the assay. To ensure that there was no bias in study conduct or in the data analysis, the study included 20 *Babesia* infection negative samples. Furthermore, the blood-film confirmed samples spanned the range of anti-*Babesia* antibody titers and parasitemia representative of the diagnostic population. Results from these known *Babesia* negative samples were not included in the calculation of sensitivity, but were for information only.

Table 15: Results of Sensitivity Study

	Smear Positive	Smear Negative
AFIA Reactive	72	0
AFIA non-Reactive	0	20

Sensitivity calculation: 100% X 72/ (0+72) = 100% (95% CI = 95.01% - 100.00%).

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

i. Performance of a proper sensitivity study comparing AFIA to microscopically confirmed parasitemia were not submitted to FDA for review. The results of the study were then included in the response to the first CR Letter.

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 all amendments, conducted a pre-license inspection and reviewed the sponsor's response to 483 observations. All review issues were resolved. The Review Committee

recommends licensure of the Imugen *Babesia microti* Arrayed Fluorescence Immunoassay.

b) Risk/ Benefit Assessment

The Imugen *Babesia microti* Arrayed Fluorescence Immunoassay (AFIA), will significantly improve blood safety by reducing transfusion transmitted *B. microti* infection, which can be fatal in susceptible recipients. Adverse events that may occur would be a false negative test result that permitted *Babesia* infected blood to be transfused or false positive results that would result in discarding healthy, usable blood and loss of the donor who would be deferred.

The clinical studies showed a high sensitivity (100%, 95%CI= 95.01% - 100.00%) indicating low probability of a false negative result. Among the more than 90,000 units of blood from endemic areas tested with the AFIA, no cases of transfusion transmitted babesiosis have occurred. The specificity measured in the clinical trial, 99.96% (95% CI = 99.94-99.97%) suggests the low probability of false positive results.

c) Recommendation for Postmarketing Activities

No postmarket studies are recommended.