

Valencia, Iliana

From: Valencia, Iliana
Sent: Thursday, January 25, 2018 6:00 PM
To: 'Wolfgang Pieken'
Cc: 'Katie Pomerantz'
Subject: STN 125588 NAT: Information request
Attachments: Babesia_NAT_Performance.docx

Dear Dr. Pieken:

We are reviewing your biologics license application BL125588 for *Babesia microti* Nucleic Acid Test. We are providing the following request for additional information to continue our review.

We had received your updated edited summary of Pre-Clinical and Clinical Studies on Dec 1, 2017. Please review the attached updated draft document and respond with your agreement, updates, or proposed corrections ASAP as an amendment to this file. Please ensure you agree with the product name and its intended use. Please submit your response via the Document Control Center (DCC).

Sincerely,

Iliana Valencia, MS, MCPM
Chief, Regulatory Project Management Staff
FDA/CBER/OBRR/IO
240-402-8444
iliana.valencia@fda.hhs.gov



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"At the intersection of differences lies the opportunity for innovation" Joel Barker

Applicant Name: Oxford Immunotec Limited

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

Established Name (common or usual name): Babesia NAT Assay / Babesia NAT Blood Screening Test

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 *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. It is also intended for use as 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.

1. Manufacturing and Controls (CMC)

a) Manufacturing Summary

The *Babesia* 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 *Babesia* NAT are either purchased from suppliers or manufactured by Imugen. Table 2 summarizes the critical materials and equipment that are used in the manufacture of the NAT device along with the supplier used.

Table 2: Suppliers of Critical Materials & Equipment of the NAT Device

(b) (4)

(b) (4)

Custom (b) (4) *Babesia* 18S rRNA primers and probes, human 18S rRNA primers and probes, and (b) (4) are purchased from (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 inspection process. The oligos are received in lyophilized form and are reconstituted by Imugen with sterile water purchased from (b) (4). Each component is release tested with the Imugen *Babesia* NAT using positive and negative controls and must meet performance specifications.

(b) (4) DNA Mini Kits are purchased from (b) (4) and the kits are tested with the *Babesia* 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 parasitemia, absence of non-*Babesia* parasites, and bacterial contamination.

Two of the critical reagents used in the *Babesia* NAT are manufactured at Imugen. Table 3 lists the components manufactured by Imugen.

Table 3: NAT Device Components Manufactured by Imugen

Component	Description
Positive NAT control (high positive and low positive)	The positive controls consist of characterized <i>Babesia</i> infected (b) (4) whole blood diluted in <i>Babesia</i> negative human whole blood to appropriate concentration levels. The controls are aliquoted into vials and release tested per LAB-MFG-10. Positive controls are used to confirm the detection of <i>B. microti</i> nucleic acid at high and low levels with <i>Babesia</i> NAT, and to confirm the extraction step.
Negative NAT control	The negative NAT control consists of

	human whole blood characterized as <i>Babesia</i> negative. The controls are aliquoted into vials and release tested per SOP LAB-MFG-12 The negative control is used to ensure there are no interferences in the NAT testing, and to confirm extraction the extraction step.
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2. 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.

5.1 Sample requirements, storage and stability

The *Babesia* NAT uses EDTA whole blood. Specimens may be stored at Imugen in the refrigerator, 2-8°C, until testing is performed. The sponsor assessed the stability of the whole blood samples for the *Babesia* 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 *Babesia* NAT negatives and 8 *Babesia* 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
<i>Babesia</i> 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. (b) (4)

5.2 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 Whole Blood, 2) High Positive Control Eluate (DNA), 3) Low Positive Control Whole Blood, and 4) Negative Control Whole Blood; and the (b) (4) components: 1) (b) (4); 2) *Babesia microti* Sequence Detection Primers; 3) (b) (4) *Babesia* Probe; 4) Human 18S Sequence Detection Primers; and 5) (b) (4) Human 18S Probe. Three Lots were manufactured,

release tested per the SOPs, and tested by the standard release test at each of the time points shown in the Table 7 below.

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.

5.3 Limit of Detection/Repeatability

Since no *B. microti* International Standard is available, the sponsor used a well characterized human clinical *Babesia* NAT positive specimen (b) (4) total *Babesia*/mL) to determine the assay's limit of detection. The NAT positive specimen was diluted into *Babesia* NAT negative human whole blood to final concentrations of (b) (4) *Babesia*/mL. Each concentration preparation was extracted in (b) (4) replicates using the (b) (4), and (b) (4)

Empirical data, presented in Table 8, indicate a (b) (4) detection limit for the NAT of approximately (b) (4) *Babesia*/mL. The actual (b) (4) detection limit was extrapolated from the data. The results (% Detection vs. *Babesia*/mL concentration) were plotted and the (b) (4) detection frequency limits were extrapolated from the graph. The (b) (4) detection frequency limit for the *Babesia* NAT was determined to be (b) (4) *Babesia*/mL as extrapolated from the graph. The (b) (4) detection frequency were determined to be (b) (4) *Babesia*/mL and (b) (4) *Babesia*/mL, respectively.

Table 8. *Babesia microti* NAT Limit of Detection

(b) (4)

5.4 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. microti* target and were tested by the *Babesia* 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*.

5.5 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	(b) (4)
<i>Hemophilus influenza</i>	
<i>Escherichia coli</i>	
<i>Pseudomonas aeruginosa</i>	
<i>Candida albicans</i>	
<i>Staphylococcus aureus</i>	
<i>Streptococcus pyogenes</i> (Group A)	
<i>Streptococcus pneumoniae</i> (pneumococcus)	
<i>Streptococcus faecalis</i> (Enterococcus)	
<i>Borrelia burgdorferi</i>	
<i>Plasmodium falciparum</i>	

(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 *Babesia* NAT with spiked and unspiked specimens. For *Plasmodium falciparum*, 20 parasite positive clinical samples were combined (b) (4) with *Babesia 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 value 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 *Babesia* NAT.

5.6 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

notebook study was run in which a positive control was prepared at a higher concentration of *Babesia*/mL, and the study was repeated on a (b) (4). This run checked for possible contamination at very high concentrations of *Babesia*. A subsequent plate 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)

3. 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 *Babesia* NAT.

Clinical specificity

The clinical specificity study #2 was designed to prospectively screen 13,192 archived blood samples collected by American Red Cross for *B. microti* by Imugen *Babesia* 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 *Babesia* NAT assay in non-endemic donors was found to be 100.00% $\{3969 / (3969 + 0) \times 100\}$ 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 AFIA. 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, enhanced PCR (ePCR), and WB (IgM and IgG). The follow-up sample drawn 12 months after the index 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 specificity of the NAT assay in donors from an endemic area (retrospective study) was found to be 99.99% $\{9189 / (9189 + 1) \times 100\}$ with 95% CI of 99.94% - 100.00% (Table 10).

Table 10: Comparison of NAT to AFIA* assay in endemic region

	AFIA Positive	AFIA Inconclusive	AFIA Negative	Total
NAT positive	7	0	0	7
NAT inconclusive	0	0	1**	1
NAT negative	24	2	9189	9215
Total	31	2	9190	9223

(*) AFIA ≥ 128 Cutoff

(**) NAT inconclusive result was initially reactive sample that was negative upon re-test.

The prospective study (Study 3a) was intended to test the specificity of the investigational tests in prospective 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, Western Blot (WB) positive and ePCR positive at index. A follow-up sample at 27 days after index 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 Wisconsin/Minnesota. Out of the 9 cases, 8 donors had at least one follow-up and 7 of them seroconverted (AFIA and WB).

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% $\{[88564 / (88564 + 1)] \times 100\}$ with 95% CI of 99.99% to 100 % (Table 11).

Table 11: 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	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.

Under 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 Performance Evaluation

Evaluation of the Sensitivity of *Babesia microti* Nucleic Acid Test

The evaluation of the sensitivity of *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 (95.01%, 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.

Clinical Reproducibility

The precision study for *Babesia microti* Nucleic Acid Test was performed under protocol Doc Pro-26 in IND #14532. Precision was assessed across the following factors:

(b) (4)

The other panels included the No Template, Negative, Low Positive and High Positive Control). Testing is summarized in Table 12.

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

(b)	(4)
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The results showed 100% agreement with expected results for all samples and controls (Table 13).

Table 13: Summary of Overall Results

