

## Valencia, Iliana

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**From:** Valencia, Iliana  
**Sent:** Monday, November 27, 2017 11:12 AM  
**To:** 'Wolfgang Pieken'  
**Cc:** 'Katie Pomerantz'  
**Subject:** BL125588 Oxford NAT: Interactive review

Oxford Immunotec, Inc Attention:  
Wolfgang Pieken, PhD Sent  
by email

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 are in the process of writing the Summary Basis of Regulatory Approval (SBRA) document. This SBRA document will completely summarize the review of the BLA and a redacted version of it will be posted on the FDA website along with notice of the final action regarding the *Babesia microti* NAT Assay once the review process is complete. We are seeking mutual agreement on the results of pre-clinical and clinical studies that will be the core of the SBRA document.

Please review the attached draft document and respond with your agreement, updates, or proposed corrections by Dec 8, 2017 as an amendment to this file. Please submit your response via the Document Control Center (DCC).



BL125588  
Summary of File: CL...

Sincerely,

Iliana Valencia, MS, MCPM  
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"At the intersection of differences lies the opportunity for innovation" Joel Barker

## 5. Analytical Studies

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

### 5.1 Sample requirements, storage and stability

The NAT assay uses EDTA whole blood. Specimens may be stored at Oxford Immunotec in the refrigerator, 2-8 C, until testing is performed. The sponsor assessed the stability of the whole blood samples for the *Babesia* NAT assay for assigning 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 were stable for at least (b) (4)

### 5.2 IVD / Kit stability

A stability study was performed to define a stability claim of each Finished Device Lot of the of *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 Finished Device Lots were manufactured and release tested per the SOPs and tested by the standard release test at each of the time points shown in the table below ()

### 5.3 Limit of Detection/Repeatability

Since no *B. microti* International Standard is available, Oxford Immunotec used a well characterized human clinical *Babesia* NAT positive specimen (b) (4) total *Babesia*/mL) into *Babesia* NAT negative human whole blood to final concentrations of (b) (4) *Babesia*/mL. Each concentration preparation was extracted (b) (4) times using the (b) (4), and each extraction was tested (b) (4) times by PCR amplification on the (b) (4). There were (b) (4) replicates for each concentration. All replicates were positive for the internal human 18S control. Empirical data, presented in Table , 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 . *Babesia microti* NAT Limit of Detection

Total Babesia	Number of	Number of	% Positive
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(b) (4)

#### 5.4 Endogenous Interferences

The sponsor assessed analytical specificity of the *Babesia microti* Nuclei Acid Test Assay 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 *Babesia microti* target and were tested by NAT assay with spiked and unspiked specimens. No interference was demonstrated in samples spiked with *Babesia microti*. There were no false negative results observed in the spiked samples.

#### 5.5 Cross-Reactivity

The assay specificity of the *Babesia microti* Nuclei Acid Test Assay 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

Table:

Organism
<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)

(b) (4) *Babesia* negative whole blood samples were spiked with (b) (4) cultured microorganisms at (b) (4) CFU/mL including *Hemophilus influenza*, *Escherichia coli*

*Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Streptococcus pyogenes* (Group A), *Streptococcus pneumoniae* (pneumococcus), *Streptococcus faecalis* (Enterococcus) and *Borrelia burgdorferi*. The samples were spiked with (b) (4) LoD of *Babesia microti* target and were tested by NAT assay 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.

### 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 (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)

### 5.7 Review issues

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

## 6. Clinical Studies

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) have been screened under the IND protocols for evidence of *B. microti*. Blood donors in regions predicted to be high endemic, low-medium endemic, and non-endemic for *B. microti* were included in the clinical study to evaluate the performance of the NAT assay. Although testing under the IND is ongoing, the dataset for the clinical specificity and sensitivity studies was closed on October 2015.

### 6.1 Clinical specificity

Under the clinical specificity study #2 prospective assessment of archived blood donor testing from American Red Cross were tested in endemic and nonendemic geographic areas. The specificity of the 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 result was observed. The specificity of the investigational NAT test in endemic donors (CT, MA, MN, WI)

was assessed by comparison to the AFIA. 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 ).

Table

	AFIA Positive	AFIA Inconclusive	AFIA Negative
NAT positive	7	0	0
NAT inconclusive	0	0	1*
NAT negative	24	2	9189

(\*) NAT inconclusive results are initially reactive samples that are negative upon re-test

The prospective donor testing was conducted with samples collected at American Red Cross (Study 3A) and Memorial Blood Centers (Study 3B) in endemic geographic areas. A total of 88,904 blood donor specimens were screened. There were a total of 62 (0.07%) NAT positive donors and 1 (0.001%) NAT inconclusive donor identified in the study. Donors who initially test NAT positive were retested in triplicate to confirm the positive finding prior to reporting a NAT positive result. The inconclusive result reported in this donor was due collectively to an initial positive result and 3 negative findings in the confirmatory replicates. The 1 NAT Inconclusive donor (MA) 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 1:64 AFIA titer at day 551 post index sample; and the titer fell below 1:64 at day 634. Nine (0.01%) window period donors identified 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 88904 donor samples tested, one donor whose index sample was NAT positive and AFIA negative with no follow up sample available was excluded from the sensitivity 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).

Table

	AFIA Positive	AFIA Inconclusive	AFIA Negative	Total
<b>NAT positive</b>	60 <sup>i</sup>	0	1 <sup>ii</sup>	61
<b>NAT inconclusive</b>	1	0	0	1
<b>NAT negative</b>	275	2	88564	88841
<b>Total</b>	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.

The frequency of NAT positive findings for the total study population in months of highest probability of recent tick-borne transmission to donors (May-November) was 0.09%, compared with 0.02% in December-April ( $p < 0.0001$ ).

Under Study 3b prospective study was conducted in Minnesota (a geographical area with low disease prevalence categorized as a low endemic area). A total of 1,187 whole blood units 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* Nuclei Acid Test

The evaluation of the sensitivity of *Babesia microti* Nuclei Acid Test was done using 72 confirmed *B. microti* blood film positive clinical samples. To avoid the bias 23 *B. microti* negative samples were included in the testing (24% of study samples). Operators were blinded to the infected status of all specimens prior to testing. The *Babesia microti* Nuclei Acid Test Assay test detected 100% of the positive samples with 95% CI (95.01%, 100.00%). 22 of the 23 negative specimens were negative by NAT, one was inconclusive. The inconclusive result had high CT value; it tested negative upon repeat testing.

### Clinical Reproducibility

The precision study for *Babesia microti* Nuclei 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 below

Table, Expected Results and Total # for NAT Precision Studies

(b) (4)

(b) (4)

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

Table Summary of Overall Results

(b) (4)

**Review Issues**

The following were the major review issues identified by the committee during review of the clinical studies and their resolution.