



Food and Drug Administration
1401 Rockville Pike
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Final Review Memorandum

BLA: STN 125589\0\12 and STN 125589\0\13

Date: 06-01-2017

To: Robert Duncan, Ph.D., Scientific Lead, LEP/DETDD/OBRR

Reviewer: Rana Nagarkatti, Ph.D., LEP/DETDD/OBRR

RPM: Iliana Valencia, MS, MCPM, Chief, RPMS, FDA/CBER/OBRR/IO

Through: Sanjai Kumar, Ph.D., Chief LEP/DETDD/OBRR

Through: Alain Debrabant, Ph.D., LEP/DETDD/OBRR

Sponsor / Product: Imugen, Inc: Blood donor screening test for evidence of *Babesia microti* (*B. microti*) by Arrayed Fluorescence Immunoassay (AFIA).

Purpose of the Submission: Response to the CR letter issued to Imugen Inc. (now Oxford Immunotec) on September 29, 2015.

Intended Use: The intended use as stated by the sponsor is, “*The Imugen B. microti AFIA (IFA) and NAT PCR are used as complementary tests for screening blood donors to determine B. microti infection as a means to reduce incidence of transfusion transmitted babesiosis*”. The final intended use statement has not been provided.

Review Discipline: Pre-clinical and Clinical sections.

Recommendation: The sponsors responses to the preclinical and clinical issues raised in the CR and a subsequent IR (dated February 24, 2017) are adequate. I recommend the approval of the BLA pending the satisfactory resolution of all issues raised in the CR that are being reviewed by the committee. In addition, the sponsor needs to provide an updated draft of the Package Insert for review and respond to the letter ready comments below.

Comments:

Brief summary of the BLA review timeline: FDA communicated a 53 item CR letter to Imugen on September 29, 2015. Imugen requested an extension of the response due date on September 16, 2016. FDA also received a partial response to

the CR letter on August 31, 2016, detailing the response to question 1 thru 6 of the issues in the clinical section of the CR letter. This partial response was reviewed by the clinical reviewers. On November 11, 2016, FDA communicated to Imugen that the response to the clinical section was adequate; however, clarifications regarding the selection of samples for determining clinical sensitivity and the total sample size for determining specificity were needed. On December 13, 2016, Imugen submitted a complete response to the CR letter. The comments below document my review of the clinical and pre-clinical sections of the sponsor's complete response and their response to the IR (received on March 20, 2017). The ADD for the submission is June 14 2017.

Principle of *B. microti* AFIA:

The *B. microti* Arrayed Fluorescence Immunoassay 'Babesia AFIA' is based on a conventional indirect immunofluorescent assay (IFA). It is used for detecting the presence of specific antibodies to *B. microti* in plasma samples from blood donors. The test uses *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 *B. microti*-specific antibody. (b) (4) fluorescence is detected by manual observation of the wells of the slide employing a microscope equipped for epi-fluorescence. Positive (human plasma containing *B. microti* antibodies) and negative controls (human plasma without *B. microti* antibodies) are used on each slide.

Clinical Issues:

CR Issue #1: *Clinical sensitivity and specificity must be calculated from all studies using the same cutoff, testing algorithm, and interpretation of results.*

a. A cutoff of 1/128 for positive detection was determined in the analytical study presented in "4-1 AFIA CMC Overview", Section 4.3.1.1, page 140. However, numerous other cut-offs were used in other studies. Briefly, in Clinical Study 1, Pedigreed Clinical Samples, results of (b) (4) were interpreted as positive, Study 2, Retrospective Donor Testing, results of 1/64 were interpreted as positive and the Prospective Studies, 3A, 3B and 4, results of 1/128 were interpreted as positive. These studies cannot be used to calculate a single sensitivity and specificity with different cutoffs. Results from Study 1 and Study 2 can only be used in evaluation of clinical performance if they are available at a 1/128 dilution; otherwise they could be used to evaluate the clinical significance of different cutoff values.

Imugen's Response: Imugen clarified that Clinical Study 1 (CS1) was exploratory and that no data from this study was used to establish the clinical sensitivity of the

assay. They submitted DOC-RPT-44 with the reanalysis of the CS1 data at 1/128 cut-off titer for the Babesia antibodies. Imugen also clarified that for the Clinical Study 2 (CS2) only specimens positive at 1/128 were used for the specificity calculations.

Comments: Clinical Study 1 was a pre-clinical study comprised of (b) (4)

A subset of 72 samples from the clinically characterized archived pedigreed samples collected in CS1 (a total of (b) (4) samples) were used to establish the clinical sensitivity of the AFIA. These were pre-selected based on NAT, however, 72 samples that were smear positive were selected and used for AFIA to calculate clinical sensitivity.

Recommendation: The CS1 does not need to be considered for BLA review as it is exploratory study. The use of 1/128 cut-off for sensitivity (CS1) and specificity (CS2) calculations are acceptable.

b. *The testing algorithm is described in LAB-SER-BIFA-1. Donor specimens are tested at an initial dilution (1/64 for retrospective, 1/128 for prospective). “All specimens reactive at a (b) (4) degree of fluorescence are repeated at the initial dilution twice and titered out to endpoint” (page 666). A donor result is reported positive if one or more of the repeat tests is positive. If both repeat tests are negative, the result is reported as negative (Table 8.4.3.5, CSR 3A, page 2849). The sponsor has not included the retest data in the spreadsheets provided. Only those samples that were positive by NAT but negative by AFIA show retest data by AFIA (CS 3A: 7 samples and CS 3B: 2 samples). In study 4, seven samples were reported positive by AFIA, but no repeat testing was recorded as required by the protocol. Please provide all retest data.*

Imugen’s Response: Imugen resubmitted the Excel file (M (MSTDONOR.xlsx) that now shows the initial and retest data in columns K through O (BCR-AFIA-ATT-3; Attachment 1.3).

Comments: The Excel file was evaluated. The sponsor has provided the retest data requested.

Recommendation: The data was acceptable.

c. *In another example of protocol deviation, the donor sample, (b) (6), in prospective study 3A was positive on index at a titer of 1/128. In 4 of the 6 follow-up samples, even though the titer was 1/64 the sample was reported as Babesia positive. A similar result was reported in study 2 for*

donor (b) (6) The sponsor must show that the testing algorithm was followed and correct interpretation was made of each test result. Alternate cutoff interpretations are not appropriate for a blood donor screening intended use. A 1/64 result should not be interpreted as “positive”. Please correct these interpretations.

Imugen’s Response: Imugen stated that the follow-up samples were tested for a secondary research study requested by the American Red Cross which was intended determine the (b) (4). This study was independent of the studies used to determine the sensitivity and specificity.

Comments: The follow up study was independent of the CS1, CS2 and CS3A (prospective screening of 89153 linked whole blood units by AFIA).

Recommendation: The response is acceptable.

CR Issue 2: In the FDA Clinical Hold Letter dated December 10 2010, FDA requested that IMUGEN “Please demonstrate the clinical sensitivity of this test in human samples that are blood-film positive for *B. microti*.” From the data provided it appears that there are approximately (b) (4) blood-film tested specimens reported in Study 1 that could possibly be used in this calculation, if they are tested at the assay cutoff of 1/128. If this is not the case then there is no calculation of clinical sensitivity presented in the submission. Please describe how clinical sensitivity will be calculated for the AFIA and any data that are included in the calculation; please submit the data as line listings in a spreadsheet.

Imugen’s Response: The sponsor stated that CS1 was not intended to provide data for the calculation of clinical sensitivity and specificity. Clinical sensitivity was calculated on 72 blood-film *B. microti* positive specimens. The 72 samples selected from CS1 archived samples were anonymized and randomized to ensure that the operators were blinded to the identity of the results of all prior Babesia testing.

Comments: The testing protocol (DOC-PRO-42; Attachment 2.1) and report (DOC-RPT-41; Attachment 2.2), were reviewed. In table 2.1 page 3 of 53, “001_AFIA Response to AI p1 to 260.pdf” document, the sponsor has provided the AFIA results for the 72 samples. Blood smear data indicated that the parasitemia for these samples ranged from 0.005% to 5.51%. The AFIA titers ranged from 128 to ≥ 1024 . As suggested by FDA, the sponsor also included 20 clinically negative samples. The 20 negative specimens were selected based on negative *B. microti* NAT clinical testing, negative serologic findings and negative blood film. All 72 out of 72 *B. microti* blood-film positive samples were AFIA positive

and all 20 out of 20 *B. microti* negative samples were AFIA negative. A clinical sensitivity of 100% was calculated based on this data.

Recommendation: The data provided was acceptable.

CR Issue 3: *To enable a claim for plasma (b) (4) specimens, data must be presented with sufficient testing of each specimen type. Please provide these data or a plan for a study. Previous submissions for blood donor screening assays have tested at least 50 sets of paired specimens with (b) (4) plasma drawn from the same donor. In addition, a sufficient number of the prospective specimens, at least 1/3 of the clinical study, should be collected as one of the sample types.*

Imugen's Response: Imugen decided that the claim for the AFIA test sample matrix will be for the use of plasma only.

Comments: All the clinical studies were performed with plasma samples collected from blood donors, thus, no additional testing is required. However, Imugen has not provided a revised package insert for review.

Recommendation: The response is acceptable. However, the sponsor needs to provide a revised package insert for review.

CR Issue 4: *The AFIA reactive donors in the clinical studies were retested with a research western blot as agreed in the IND. Please submit the complete description and validation of the western blot method including images of positive and negative test results.*

Imugen's Response: Imugen has provided the description and validation information for the western blot method, including representative images of positive and negative results (BCR-AFIA-ATT-6; Attachment 4.1) with experimental details provided in SOP LAB-SER-BWB-2.

Comments: The information presented on the western blot is acceptable. Sufficiently detailed validation has been submitted. The specificity and sensitivity was established in 2008 in a cohort of (b) (4) Babesia PCR negative and a cohort of (b) (4) PCR positive patients from southeastern Massachusetts, an area at that presumed to be of low endemicity, against the same laboratory developed PCR assay that was used in Clinical Study 1.

For the IgG *B. microti* western blot a sensitivity of (b) (4) and a specificity of (b) (4) was found.

For the combination of the IgM and IgG *B. microti* western blot a sensitivity of (b) (4) and a specificity of (b) (4) was found.

In a cohort of (b) (4) smear positive samples a sensitivity of (b) (4) was observed for the IgM *B. microti* western blot (Table 4) and a sensitivity of (b) (4) was observed for the IgG *B. microti* western blot.

Recommendation: The response is acceptable.

CR Issue 6: *Please submit a data summary for each clinical study, display the data as a 2X2 table with results for the test under review in rows and the results of the comparator test in columns. In cases where there are 3 outcomes (positive, negative, inconclusive), the data may be displayed in 2X3 or 3X3 tables.*

Imugen's Response: Imugen has provided the data in the requested format.

Comments: The organization and analysis of clinical study results in 2x2 or 3x3 tables is acceptable and substantially improves the presentation of the clinical trial results.

Recommendation: The response is acceptable.

CR Issue 7: *In the submission document, "4-1 AFIA CMC Overview," Section 4.3.1.1, you present an analytical sensitivity/cutoff study with (b) (4) blood smear positive or PCR positive diagnostic patient samples. In your conclusion you state, "The data indicate that an AFIA cutoff at a dilution of 1:128 is sufficient for detecting exposure to *B. microti*." Based on this analysis, 1:128 should be used as the cutoff in all the studies presented. Among the pre-clinical and clinical studies, (b) (4) and 1:64 were also used as cutoffs. Please perform the analysis of all studies with the 1:128 cut off.*

Imugen's Response: Imugen has provided a revised Excel document with 1:128 as the cut off.

Comments: No comments.

Recommendation: The response is acceptable.

Pre-Clinical Issues:

CR Issue 12: *At the conclusion of the Microbial Cross-Reactivity study (4-1 AFIA CMC Overview, pages 141-145) you propose repeating the study. Please provide the results of the repeat study.*

Imugen's Response: Imugen has provided additional data in DOC-RPT-35.

Comments: Imugen used Babesia negative and positive plasma samples that were spiked with 8 different bacterial species. There was no interference detected in the AFIA as Babesia negative samples remained negative after spiking and Babesia positive samples remained positive after spiking. Imugen also concluded from this study that there was no cross reactivity to bacteria. This issue was raised in an IR letter sent to Imugen on February 24, 2017. Imugen responded on March 20, 2017 and submitted an updated report "Attachement_2.1-DOC-RPT-35&DocDetails.pdf". The conclusion of this report was modified from the earlier version to state that "*The addition of the microbial organisms, listed in this report, to B. microti negative and B. microti positive spiked samples had no effect on the AFIA or NAT test systems*". Imugen provided updated documents for the protocol (Attachment 2.2; DOC-PRO-28, "Microbiological Cross Contamination Study of AFIA and NAT" and Attachment 2.3 for LAB-SER-BIFA-1 for the AFIA SOP).

Recommendation: The response is acceptable.

CR Issue 13: *In the pre-clinical studies, you showed that plasma from Plasmodium falciparum infected individuals reacts 100% (4 of 4) in the B. microti AFIA. Given that this is a significant cross reactivity that will likely be included in the labeling of this test; we recommend that at least 20 more P. falciparum infected specimens be tested to determine the specificity of the Babesia assay and the results submitted for review.*

Imugen's Response: Imugen responded that a new Plasmodium falciparum (P. falciparum) cross-reactivity study was performed (DOCPRO-43 (Attachment 13.1) and DOC-RPT-59 (Attachment 13.2)). All 20 specimens tested negative in this new study.

Comments: Imugen obtained 20 additional P. falciparum samples from (b) (4). All of these were negative on the AFIA. The original 4 P. falciparum samples were obtained from (b) (4) and as per Imugen's response to the IR, did not come with a specimen sheet. Of these four samples, three samples were positive at 1:128, 1 sample was negative at 1:128. Due to this Imugen wants to withhold the earlier cross reactivity data based on the (b) (4) samples and claim 0% cross reactivity based on the (b) (4) samples. However, it is not appropriate to reject the earlier (b) (4) samples from the cross reactivity study. There is no evidence to

support exclusion of the data from the (b) (4) samples. Thus, using a cutoff of 1:128, a cross reactivity rate of 12.5% should be calculated (3/24).

Recommendation: The response is acceptable. However, the package insert should indicate the AFIA cross reactivity rate of 12.5% with *P. falciparum* at 1:128 cut off.

CR Issue 14: *In the 4-1 AFIA CMC Overview, Table 4.3.13, the study describing endogenous potentially interfering substances, the AFIA assay produced a positive reaction in 3 out of 20 (15%) of the Anti-nuclear antibody (ANA) specimens. This appears to be high since ANA antibodies can exist in a broad range of conditions including autoimmune disorders and has a prevalence of 5% in normal individuals. Therefore, this could represent a potential confounding factor on the AFIA results. The potential reactivity with ANA antibodies will be listed as a limitation of the assay unless you can provide additional results or interpretation.*

Imugen's Response: Imugen tested an additional cohort of 20 ANA positive samples on the AFIA. All 20 samples were negative on the AFIA. Imugen concluded that there was no interference in the AFIA with the ANA antibodies.

Comments: The earlier ANA interference study indicated that 3 out of 20 ANA positive individuals were positive on the AFIA at 1:64. The sponsor needs to clarify if these 3 individuals were also positive at the cut off of 1:128. All these samples were recruited from a private practice physician in the *B. microti* endemic regions of South Eastern Massachusetts. The new cohort of 20 samples were sourced from (b) (4). However, the geographical area from where these 20 samples were collected is unknown. There is no evidence to support exclusion of samples in the first cohort. Thus, the percent interference needs to be calculated as 3/40 (7.5%).

Recommendation: The sponsor needs to clarify if the 3 ANA positive samples were AFIA positive at the 1:128 cut off. The percent interference needs to be calculated over a total of 40 samples that were tested.

Letter ready comments:

1. Please provide an updated draft of the Package Insert.
2. In the documents for the *P. falciparum* cross reactivity study, *DOCPRO-43 (Attachment 13.1)* and *DOC-RPT-59 (Attachment 13.2)*, submitted in response to the IR, 20 additional samples obtained from (b) (4) tested negative on the AFIA. Of the four samples from (b) (4) three samples were positive at 1:128. Thus, using a cutoff of 1:128, a cross reactivity rate of 12.5%

should be calculated (3/24). Please report the *P. falciparum* cross reactivity rate as 3/24 (12.5%) in the revised package insert.

3. In the ANA interference study, (*DOC-PRO-49 and DOC-RPT-71*), please indicate if the 3 ANA positive samples that were AFIA positive at a 1:64 cut off remained positive at a 1:128 cut off. Based on the number of ANA positive samples that are positive at the AFIA cut off of 1:128, please recalculate the percent interference over a denominator of 40 (i.e., the total number of ANA positive samples tested using the AFIA).