



Review Memorandum

BLA: BL125589

Date: 2/15/2018

Reviewer: Sreenivas Gannavaram, Ph.D., OBRR/DETTD/LEP

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

RPM: Alisha Miller,

Sponsor: Oxford Immunotec Ltd. (Formerly Imugen)

Product: Arrayed Fluorescence Immunoassay

Recommendation: Approval

Intended Use: The *Babesia* AFIA blood screening assay is an in vitro diagnostic test intended to permit analysis of blood donor specimens collected using EDTA tubes and delivered. Imugen's CLIA certified laboratory will perform the analysis of these samples using an arrayed fluorescence immunoassay method to detect the presence of *Babesia* antibody in the donated blood samples using *B. microti* organisms in whole blood obtained from an infected (b) (4) affixed on glass slides. Detection of the antibody bound to the *Babesia* antigen on the glass slides will be done by using (b) (4) anti-human IgG heavy and light chain specific (b) (4) conjugated antibody. The fluorescence will be detected with a microscope equipped with an epi-fluorescence illumination.

Documents Reviewed:

Chemistry manufacturing and controls including device description and back ground, attachments describing production of glass slides containing *Babesia* infected red blood cells, analytical precision studies, stability studies, endogenous substances study.

Comments:

Imugen AFIA BLA Gannavaram Memo

Device Description and background Part I:

1) The source material for the production of glass slides containing *Babesia* infected red blood

cells is obtained from the *Babesia* infected (b) (4). Infection of the (b) (4) and collection of the source material is performed at the (b) (4)

(b) (4), as a

(b) (4) source. The material obtained from the infected (b) (4) is analyzed by Genotyping to ascertain the molecular identify of the *Babesia microti* by analyzing (b) (4) different loci on the genome.

It is not clear from the submission how often the genotyping of the source material performed. Are the *Babesia microti* organisms in the starting material (infected (b) (4) blood) obtained in the field by the (b) (4)? Similarly, is the material obtained from (b) (4) as a (b) (4) source genotyped? How is the genetic identify of the *Babesia microti* organisms used in preparing the AFIA slides for the donor blood screening assay ascertained? This demonstration is important since *Babesia microti* organisms used as the source material appear to be (b) (4) animal models to maintain virulence. Periodic assessment of the genetic makeup of the parasites is essential. Therefore the genetic stability of the *Babesia* organisms used as source material is an important consideration.

In the Figure 4.2.3 flow chart that describes the processing and testing *Babesia microti* infected red blood cells at IMUGEN, only the (b) (4), absence of bacterial contamination are tested for but no further testing on the genetic identity of the *Babesia microti* deposited on the slides.

Please explain what steps are taken to ascertain the genetic stability of the *Babesia microti* organisms in infected RBC used in preparing the test material for blood screening assay.

2) In the acceptance criteria for *Babesia microti* infected red blood cells (LAB-MFG-1) described in Figure 4.2.3, the sponsor indicates that the red blood cell must have (b) (4) (b) (4) However in the Table 4.2.5 describing the manufactured components and specifications, for the B. *microti* AFIA system, (using method described in LAB-MFG-1) the

specifications call for a (b) (4). Please explain the inconsistency in the protocols

since scoring based on fluorescence upon reaction with *Babesia microti* infected serum critically depends on the amount of (b) (4).

3) According to the LAB-MFG-(b) (4) of the (b) (4) RBC suspension is added to the wells on the (b) (4) well glass slide. However, according to LAB-MFG-15 that describes specifications for AFIA slides, the antigen coverage on the wells on glass slides should be greater than or equal to (b) (4) surface. Further, the antigen coverage is to be ascertained by (b) (4)

(b) (4) Since the wells on the glass slide are of (b) (4) diameter, would the (b) (4) volume sufficient to cover the surface area of the well? Could depositing slightly more volume on the glass slide help in more fuller coverage of antigen on the wells and thus make the (b) (4) of the antigen coverage on the wells consistent? Please explain how a more robust verification of the coverage of the slides with processed (b) (4) RBCs can be achieved.

4) Table 4.3.8. In testing of samples containing potentially interfering disease states, malaria by *Plasmodium falciparum* infection always produced a positive reaction on the AFIA assay indicating that falciparum malaria is a confounding factor. Similarly, serum positive for antibodies for Chagas disease, Toxoplasma and Cytomegalovirus produced 3-6% positive reaction on AFIA. Sponsor suggests that some of the reactivity could be indicative of a previous *B. microti* exposure since the samples were collected in an endemic area and not due to the interfering substances. This seems unlikely to account for up to 6% reactivity on AFIA assay. Please explain whether this level of reactivity be due to small sample size tested.

5) Table 4.3.13. In the study describing endogenous potentially interfering substances, AFIA assay produced a positive reaction in 3 out of 20 (15%) positive reaction in Anti-nuclear antibody (ANA) test. This appears to be high since ANA antibodies can exist in a broad range of conditions including autoimmune disorders including systemic lupus erythematosus,

Sjögren's syndrome, scleroderma, mixed connective tissue disease, polymyositis, dermatomyositis, autoimmune hepatitis and drug induced lupus. Up to 20% of the population can show titers of 1:160 on an ANA assay, therefore this could be potentially a confounding factor on the AFIA results. Please explain what steps could be taken to address this confounding factor.

6) (b) (4) procedure for determining presence of (b) (4) bacteria in Babesia infected (b) (4) blood: (LAB-MFG-25). The sponsor indicates that all the new lots of (b) (4) blood will be tested for bacterial contamination by (b) (4) procedure. (b) (4)

(b) (4) to reveal the presence of bacteria. Any positive sample would be verified by a (b) (4) laboratory technologist.

Since (b) (4) blood represents the primary source material, a more rigorous microbiological examination of the source material is desirable. For example, fungal contamination is common in (b) (4) derived preparations. The procedures designed only capture bacterial contamination. Moreover the testing is done on (b) (4) according to LAB-MFG-25 that may not reveal non-bacterial contamination. Please explain what steps could be taken to assess potential non-bacterial contaminants on the glass slides to be used in the blood screening assay. *Comments following the complete response from the sponsor:*

Following the complete response provided by the sponsor, the issues raised in my initial review have been resolved.

