

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**125387Orig1s000**

**MICROBIOLOGY REVIEW(S)**



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Center for Drug Evaluation and Research  
WO Bldg 51  
10903 New Hampshire Avenue  
Silver Spring, MD 20993

**Date:** 11 October 2011  
**To:** Administrative File, STN 125387  
**From:** Colleen Thomas, Ph.D., Reviewer, CDER/OC/OMPQ/DGMPA/BMAB  
Reyes Candau-Chacon, Ph.D., Reviewer, CDER/OC/OMPQ/DGMPA/BMAB  
**Endorsed:** Patricia Hughes, Ph.D., Acting Branch Chief, CDER/OC/OMPQ/DGMPA/BMAB  
**Subject:** Amendment to original BLA for aflibercept ophthalmic solution.  
**US License:** 1760  
**Applicant:** Regeneron Pharmaceuticals, Inc.  
**Facilities:** (b) (4)  
**Product:** Eylea® (aflibercept ophthalmic solution)  
**Indication:** Neovascular (wet) age-related macular degeneration  
**Dosage:** The drug product is a sterile, preservative-free aqueous solution supplied in single-use vials. The recommended dose is 2 mg (50 microliters) of 40 mg/ml drug product administered by intravitreal injection once monthly for the first three months of treatment and then once every two months.  
**Due date:** 18 November 2011

**Recommendation for approvability:** The BLA, as amended, was reviewed from a product quality microbiology and sterility assurance perspective and is recommended for approval. There is one product quality microbiology PMC listed at the end of this memo.

## Summary

Regeneron Pharmaceuticals, Inc. submitted BLA 125387 to license aflibercept and the associated drug substance and drug product manufacturing processes. The drug product (DP) is administered by intravitreal injection and is supplied as a sterile, preservative-free solution. The original BLA included DP supplied as (b) (4)

(b) (4)

(b) (4)

(b) (4) Product quality deficiencies identified by the BMAB and OBP reviewers were communicated to the sponsor prior to the action date. The sponsor then (b) (4) responded to the deficiencies applicable to the (b) (4) vial DP. The review clock was extended to 18-Nov-2011.

Product quality microbiology information up to and including amendment 0024 was reviewed in the BMAB memo dated 3-Aug-2011, which has already been endorsed and provided to OND. The current memo covers product quality microbiology information provided in the following amendments:

- 0028 (20-Jul-2011) - stability update (b) (4) vials only
- 0029 (1-Aug-2011) - (b) (4)
- 0031 (10-Aug-2011) - (b) (4)
- 0032 (12-Aug-2011) - response to deficiencies identified by BMAB and OBP
- 0034 (1-Sep-2011) - response to BMAB information request
- 0037 (20-Sep-2011) - response to BMAB information request
- 0038 (26 Sep 2011) - response to OBP information request (section 3.2.P.3.1 only)

The following CDER/OC/OMPQ/DGMPA/BMAB reviewers contributed to this memo as follows:

- Reyes Candau-Chacon, Ph.D. - container closure integrity testing
- Colleen Thomas, Ph.D. - all other topics

Product quality microbiology review of manufacturing steps performed at the drug substance manufacturing facility (Regeneron) is covered separately in the review memo by Dr. Kalavati Suvarna, CDER/OC/OMPQ/DGMPA/BMAB.

**Drug Product Review: (b) (4) Vials**

**P.2 Pharmaceutical Development**

**P.2.5 Microbiological Attributes**

**CCI Validation: Filling Speed and Crimping Forces**

**Reviewer's question:**

Indicate whether container closure integrity has been validated using worst-case filling speed and crimping forces.

**Response summary (0034)**

The container closure integrity (CCI) was validated using a worst-case filling speed and a standard seal crimping force. The filling of the batches from which the samples for the CCI testing were taken was conducted at the maximum speed setting for the EYLEA filling line. The crimping of the vial seals used a force of (b) (4) which is the set point for EYLEA vials. The combination of worst-case vial filling speed with the routine crimping force is the established (b) (4) approach for validating vial CCI.

**Reviewer's question:**

Container closure integrity was validated using worst case filling speed (maximum speed). However, validation took place using routine crimping forces. Please validate container closure integrity using min/max crimping forces. Alternatively, justify why min/max crimping forces cannot be used for container closure integrity validation.

**Response summary (0037)**

(b) (4) validated vial container-closure integrity using minimal (b) (4) and maximal (b) (4) crimping forces. 200 vials were validated for each crimping force using the dye ingress test and the conditions and controls described in the original BLA.

SATISFACTORY

**CCI Validation: Microbial Ingress Test**

**Reviewer's question:**

Provide the bacterial concentration at the end of the microbial ingress test.

**Response summary (0034)**

Container closure integrity was assessed by submersing vials for 30 minutes at -0.3 bar, 30 minutes at atmospheric pressure, and 30 minutes at +0.2 bar in a bacterial suspension containing *Brevundimonas diminuta* and *Staphylococcus epidermis*. The total bacterial concentration of the suspension determined following the submersion of the vials was  $5.6 \times 10^8$  CFU/mL.

**Reviewer's comment:**

The bacterial concentration in the suspension ( $5.6 \times 10^8$  CFU/mL) provided sufficient challenge for the microbial ingress test.

SATISFACTORY

**P.3.1 Manufacturers**

Drug product manufacturer information, as updated in amendment 0038, is shown in the tables below.

**Drug Product Manufacturers**

Site Name:	Regeneron Pharmaceuticals, Inc.
Address:	(b) (4)
Contact:	(b) (4)
Telephone Number:	
Drug Master File Number:	
Establishment Number:	
Manufacturing Process Step(s):	(b) (4)
Type of Testing	(b) (4)
Site Inspection	PAI performed May 2011
Site Name:	(b) (4)
Address:	
Contact:	
Telephone Number:	
Drug Master File Number:	
Establishment Number:	
Manufacturing Process Step(s):	
Type of Testing	
Site Inspection	

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**Drug Product Manufacturers**

(b) (4)

**P.3.5 Process Validation**

**Hold Time Validation**

**Hold Time Definition**

**Reviewer's question:**

[Redacted] (b) (4)  
[Redacted]

**Response summary (0032)**

[Redacted] (b) (4)  
[Redacted]

**Hold Time Validation**

**Reviewer's question:**

Regarding hold time validation studies performed at [Redacted] (b) (4), validation of hold times for microbial control at scale is facility-specific. Therefore, hold time studies performed at the [Redacted] (b) (4) syringe manufacturing site do not support hold time validation at the [Redacted] (b) (4) vial manufacturing site. Provide at scale end of hold bioburden and endotoxin data from three lots of drug product manufactured at the [Redacted] (b) (4) vial site.

**Response summary (0032)**

Regeneron commits to validating the hold time for three additional lots of drug product in vials manufactured at (b) (4) and providing end of hold bioburden and endotoxin data for these lots. The validation should be completed and the results submitted to the agency within one year after BLA approval.

**Reviewer's comment:** Hold time validation will be a PMC. The results should be provided in a CBE-0 supplement.

SATISFACTORY (PMC)

**Sterilizing Filter Validation**

**Microbial Retention**

**Reviewer's question:**

(b) (4)

(b) (4)

(b) (4)

(b) (4)

SATISFACTORY

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SATISFACTORY

**Conclusion**

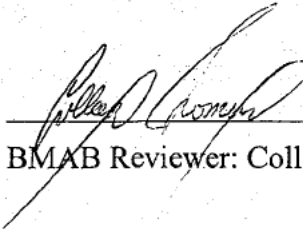
- I. The BLA, as amended, was reviewed from a product quality microbiology perspective and is recommended for approval. There is one product quality microbiology PMC.
- II. Product quality aspects other than microbiology should be reviewed by OBP.
- III. The pre-license inspection for the drug product manufacturing facility was waived. The inspection waiver memo is attached.

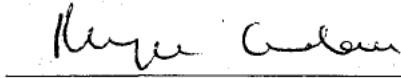


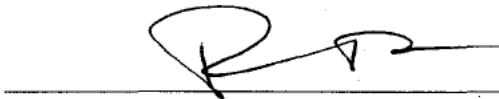
### **Microbiology Product Quality PMC**

Perform at-scale hold time validation studies with three lots of 40 mg/ml aflibercept manufactured at the (b)(4) vial site. Include bioburden and endotoxin data from samples taken at the end of the hold times. Provide the results in a CBE-0 supplement within one year of BLA approval.

**Signatures and Distribution List**

  
BMAB Reviewer: Colleen Thomas, Ph.D. 13 Oct 2011  
Date

  
BMAB Reviewer: Reyes Candau-Chacon, Ph.D. 13/10/2011  
Date

  
Concurring BMAB Acting Branch Chief: Patricia Hughes, Ph.D. 10/13/11  
Date

CC: OMPQ/BMAB/Building 51, Thomas  
OMPQ/BMAB/Building 51, Candau-Chacon  
OMPQ/BMAB/Building 51, Hughes  
HFD-520, Puglisi, Michael  
OMPQ/BMAB/Building 51, eCTD Files (STN:125387)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration  
Center for Drug Evaluation and Research  
WO Bldg 51  
10903 New Hampshire Avenue  
Silver Spring, MD 20993

**Date:** 3 August 2011  
**To:** Administrative File, STN 125387  
**From:** Colleen Thomas, Ph.D., Reviewer, CDER/OC/OMPQ/DGMPA/BMAB  
Reyes Candau-Chacon, Ph.D., Reviewer, CDER/OC/OMPQ/DGMPA/BMAB  
**Endorsed:** Patricia Hughes, Ph.D., Acting Branch Chief, CDER/OC/OMPQ/DGMPA/BMAB  
**Subject:** Original BLA for aflibercept ophthalmic solution.  
**US License:** 1760  
**Applicant:** Regeneron Pharmaceuticals, Inc.  
**Facilities:** [REDACTED] (b) (4)  
[REDACTED]  
[REDACTED]  
**Product:** Aflibercept  
**Indication:** Neovascular (wet) age-related macular degeneration  
**Dosage:** The drug product is a sterile, preservative-free aqueous solution supplied in single-use vials [REDACTED] (b) (4). The recommended dose is 2 mg (50 microliters) of 40 mg/ml drug product administered by intravitreal injection once monthly for the first three months of treatment and then once every two months.  
**Due date:** 20 August 2011

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**Recommendation for approvability:** The BLA was reviewed from a product quality microbiology and sterility assurance perspective and is not recommended for approval. Product quality microbiology deficiencies for each drug product presentation are listed at the end of this review.

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## **Summary**

Regeneron Pharmaceuticals, Inc. submitted BLA 125387 to license aflibercept and the associated drug substance and drug product manufacturing processes. Aflibercept ophthalmic solution is indicated for treatment of neovascular age-related macular degeneration. Aflibercept is a recombinant protein produced in CHO cells. It contains sequences from human vascular

endothelial growth factor (VEGF) receptor domains fused to the Fc portion of human IgG1. The drug product is administered by intravitreal injection and is supplied as a sterile, preservative-free solution in (b) (4). Vials are single-use and contain (b) (4) 40 mg/ml aflibercept.

The scope of this review is product quality microbiology information provided for (b) (4) drug product vials manufactured at (b) (4). The CDER/OC/OMPQ/DGMPA/BMAB reviewers contributed to this memo as follows:

- Reyes Candau-Chacon, Ph.D. - sections 3.2.P.2.5 (container closure integrity)
- Colleen Thomas, Ph.D. - all other sections

Product quality microbiology information provided for manufacturing steps performed at the drug substance manufacturing facility (including the formulation step) is reviewed separately by Dr. Kalavati Suvarna, CDER/OC/OMPQ/DGMPA/BMAB.

The majority of the product quality microbiology information for the drug product (DP) presentations was not submitted with the original BLA and was obtained from the following amendments:

- 0004 (24-Mar-2011) - DMF LOAs
- (b) (4)
- 0009 (11-Apr-2011) - (b) (4) vial DP process validation
- (b) (4)

Amendment 0024 (5-Jul-2011) was submitted in response to an information request from OBP. Some of the process validation information provided in amendment 0024 was also included in amendments 0007, 0009, and 0010. The new product quality microbiology information was limited to the responses for questions 18(d), 20(b)(ix), 22, 24(a), and 24(b). This information was included in this review.

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## P.8 Stability

The proposed shelf life for DP in vials is (b) (4) at 2-8°C protected from light. Stability test results for endotoxin and sterility assurance (beyond t = 0) are not yet available for conformance lot samples at the recommended storage condition. A stability update was provided on 20-Jul-2011 but will not be reviewed during this review cycle.

### P.8.2 Post-Approval Stability Protocol and Stability Commitment

The post-approval stability commitment and stability test schedule is the same as for (b) (4) vials.

**Reviewer's comment:** See comments under (b) (4) vial review.

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## **Additional Information**

### **3.2.A Facilities and Equipment**

General facility information is included under review of each drug product. Section 3.2.A provides additional information for each drug product manufacturing facility, including floor plans and flow diagrams.

#### **Module 1: Package Insert**

The dosage forms and strengths include the 40 mg/ml vial (b) (4); presentations for intravitreal injection. (b) (4). The package insert states that the DP should be inspected visually prior to usage and the vial (b) (4) should not be used if there is visible particulate matter, cloudiness, or discoloration. (b) (4)

(b) (4)

#### **Module 1: Environmental Assessment**

A claim for a categorical exclusion from preparing an Environmental Assessment under 21 CFR 25.31(b) was provided by the sponsor on the grounds that the estimated concentration of the substance at the point of entry into the aquatic environment will be below 1 part per billion annually. The sponsor provided calculations that included anticipated production of the DP over the next five years for commercial use under BLA 125387. (b) (4)

(b) (4)

#### **CGMP Status**

The following update on the CGMP status of the drug product manufacturing facilities was provided by OMPQ/NDMAB on 27-Jul-2011. There are no pending or ongoing compliance actions that would prevent approval of this application.

#### **Drug product manufacturing (vials) and sterility testing**

(b) (4)

(b) (4)

(b) (4)

#### **Drug product manufacturing (vials) and sterility testing**

(b) (4)

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## Conclusion

- I. The BLA was reviewed from a product quality microbiology perspective and is not recommended for approval.
- II. Product quality aspects other than microbiology should be reviewed by OBP.
- III. Pre-license inspections for the drug product manufacturing facilities were waived. As indicated under CGMP status, the facilities were recently inspected by ORA for therapeutic biologic drug product manufacturing and found acceptable.

**Microbiology Product Quality Deficiencies**

**Product Quality Microbiology Deficiencies (Drug Product)**



(b) (4)





8. Regarding container closure integrity testing of drug product in vials:



- b. Indicate whether container closure integrity has been validated for the (b) (4) and (b) (4) vials using worst-case filling speed and crimping forces.
- c. Provide the bacterial concentration at the end of the microbial ingress tests performed for the (b) (4) and (b) (4) vials.



9.



10. Regarding media fills performed at (b) (4)

- a. Indicate which steps of a production fill were simulated during media fills performed in (b) (4)
- b. Provide a summary of the environmental monitoring results obtained during the media fills. Include information on the number of samples collected and the number of samples exceeding the action limits in (b) (4)  
[REDACTED]

[REDACTED] (b) (4)

[REDACTED] (b) (4)

[REDACTED] (b) (4)

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**Product Quality Microbiology Possible PMCs (Drug Product)**

1. Performance of the container closure integrity test in lieu of the sterility test for drug product stability samples at expiry is recommended.

[REDACTED] (b) (4)

3. Regarding hold time validation studies performed at [REDACTED] (b) (4) hold time studies for microbial control at scale is facility-specific and should be performed for each facility even if the processes are the same and identical equipment is used. Provide at scale end of hold bioburden and endotoxin data from three lots of drug product manufactured at the [REDACTED] (b) (4) vial site and from one additional lot of drug product manufactured at the [REDACTED] (b) (4) syringe site.

**Product Quality Microbiology Clarification Questions (Drug Product)**

1. Indicate whether the endotoxin release testing of the finished drug product will employ a 1:8 dilution or other dilution below the MVD.
2. A discrepancy is noted in the BLA for [REDACTED] (b) (4) vial manufacturing. Indicate whether the [REDACTED] (b) (4) hold time is until the end of filling (as indicated in 3.2.P.3.3) or until the start of filling (as indicated in section 3.2.P.3.5).


**Signatures and Distribution List**

BMAB Reviewer: Colleen Thomas, Ph.D.



Date: 4 Aug 2011

BMAB Reviewer: Reyes Candau-Chacon, Ph.D.



Date: 8/4/2011

Concurring BMAB Acting Branch Chief: Patricia Hughes, Ph.D.



Date:

8/4/2011

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OMPQ/BMAB/Building 51, Candau-Chacon

OMPQ/BMAB/Building 51, Hughes

HFD-520, Puglisi, Michael

OMPQ/BMAB/Building 51, eCTD Files (STN:125387)

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U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research  
Office of Compliance  
Office of Manufacturing and Product Quality  
Biotech Manufacturing and Assessment Branch

## PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

**REVIEWER:** Kalavati Suvarna, Ph.D.  
**ACTING BRANCH CHIEF:** Patricia Hughes, Ph.D.

<b>BLA</b>	125387/0
<b>APPLICANT</b>	Regeneron Pharmaceuticals, Inc.
<b>US LICENSE NUMBER</b>	1760
<b>SUBMISSION REVIEWED</b>	Original BLA
<b>PRODUCT</b>	EYLEA (Aflibercept; BAY 86-5321)
<b>MANUFACTURING FACILITY</b>	(b) (4)
<b>INDICATION</b>	Treatment of Neovascular (Wet) Age-Related Macular Degeneration
<b>DOSAGE FORM</b>	Solution for intravitreal injection (40 mg/mL (b) (4) vials and (b) (4)
<b>SUPPORTING DOCUMENTS</b>	IND 12462
<b>CDER RECEIPT DATE</b>	February 18, 2011
<b>REVIEW ASSIGN DATE</b>	February 24, 2011
<b>REVIEW COMPLETE DATE</b>	July 22, 2011
<b>GRMP GOAL DATE</b>	July 23, 2011
<b>PDUFA GOAL DATE</b>	August 20, 2011
<b>PROJECT MANAGER</b>	Michael Puglisi
<b>DIVISION</b>	Division of Anti-Infective and Ophthalmology Products
<b>TO</b>	S:\archive\BLA\125387\STN125387.rev.mem.BLA.06-23-2011.doc

## I. PRODUCT QUALITY MICROBIOLOGY SUMMARY

### I. EXECUTIVE SUMMARY

The subject of this BLA is aflibercept, a recombinant protein consisting of sequences derived from the human vascular endothelial growth factor (VEGF) receptor extracellular domains fused to the Fc portion of human immunoglobulin G1 (IgG1). Aflibercept acts as a soluble decoy receptor that binds VEGF-A and PlGF, thereby inhibiting the binding and activation of these cognate VEGF receptors. (b) (4)

(b) (4) The final formulated product is referred to as VEGF Trap-Eye or aflibercept ophthalmic solution and is available in (b) (4) single-dose vials (b) (4)

(b) (4) This review covers microbial control of the drug substance manufacturing process described in the original BLA and amendments (eCTD sequences 0002 dated 3/10/2011, 0006 dated 4/4/2011, and 0011 dated 4/13/2011, 0015 dated 5/23/2011, 0018 dated 6/7/2011, and 0023 dated 6/29/2011). For a review of the microbial controls in drug product manufacture and sterility assurance of drug product, please see the review by Dr. Colleen Thomas. The drug substance is manufactured at (b) (4)

(b) (4) The site was inspected from (b) (4) and the inspection has been initially classified as VAI. (b) (4)

(b) (4) treatment regimen for VEGF Trap-Eye is 2 mg (50 µL) administered by intravitreal injection once every 2 months, following 3 initial monthly injections of 2 mg (50 µL).

### II. Recommendation and Conclusion on Approvability

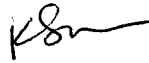
Sections 3.2.S of the BLA pertaining to microbial control of the drug substance manufacturing process were reviewed. The BLA, as amended, is recommended for approval from a CMC microbiology product quality perspective. (b) (4)

(b) (4) were inspected by a team of investigators from (b) (4) A 2-item FDA form 483 was issued at the end of inspection. A recommendation was made to classify this inspection as VAI. The final inspection outcome is pending with Office of Compliance.

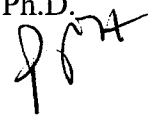
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**SIGNATURES/DISTRIBUTION LIST**

Primary BMAB Reviewer: Kalavati Suvarna, Ph.D.



Concurring BMAB Acting Branch Chief: Patricia F. Hughes, Ph.D.



Date: 7/22/2011

Date: 7/29/2011

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- HFD-590/ Puglisi, Michael
- Building 51/Hughes, Patricia
- HFD-590/Wadhwa, Sonal
- HFD-590/Boyd, Williams