



**Department of Health and Human Services  
Public Health Service  
Food and Drug Administration  
Center for Biologics Evaluation and Research**

---

**To:** BLA STN 125325\0 File

**From:** Maria L. Virata-Theimer, Ph.D., LPD/DH/OBRR, HFM-345

**Through:** Dorothy E. Scott, M.D., Chief, LPD/DH/OBRR, HFM-345  
Mei-ying W. Yu, Ph.D., LPD/DH/OBRR, HFM-345

**CC:** Cherie Ward-Peralta, RPM, DBA/OBRR, HFM-380

**Applicant:** Kamada Ltd., Ness-Ziona, Israel

**Product:** Alpha-1-Proteinase Inhibitor (Human)  
Proposed Trade name: Glassia™

**Subject:** Final CMC Review: Original BLA – Sterility, Pyrogen, Bacterial Endotoxin, General Safety Testing

---

### **Executive Summary**

This Final Review memorandum covers specific assigned CMC sections of the original Biologics License Application (BLA) submission from Kamada Ltd. for Alpha-1-Proteinase Inhibitor (Human) (Glassia™) (Kamada-API or AAT), which was received by FDA CBER on 1-JUN-09. The CMC sections that I reviewed were on sterility, pyrogen, bacterial endotoxin, and General Safety testing. The method validation results and proposed specifications for these tests were found to be acceptable.

- **Sterility testing:** To test for sterility, Kamada utilizes a membrane filtration method (that uses the ---(b)(4)--- column system), which is based on -----(b)(4)----- and -----(b)(4)----- . The sterility specifications for the bulk and the final container material are both listed as “Pass”, meaning “sterile”.
- **Pyrogen testing:** -----(b)(4)-----, an approved contract laboratory, performs the rabbit pyrogen test for lot release and stability testing of Kamada-API, according to -----(b)(4)----- . The specification is listed as “Pass”.
- **Bacterial endotoxin testing:** To test for bacterial endotoxins, Kamada uses a -----(b)(4)-----, based on -----(b)(4)----- . Reagents and control standards are from ----(b)(4)---- -----, while the final product (lot release) specification is set at “----- (b)(4) -----”.
- **General Safety testing:** -----(b)(4)-----, an approved contract laboratory, performs the General Safety test for lot release of Kamada-API using mice and guinea pigs, according to 21 CFR 610.11. The General Safety test specification is listed as “Meets requirements”.

### **Recommendation**

The specific assigned CMC sections on sterility, pyrogen, endotoxin and General Safety testing were found to be acceptable. There are no further issues.

## **Background Summary**

FDA CBER received on 1-JUN-09 this original Biologics License Application (BLA) submission (dated 29-MAY-09) from Kamada Ltd. for Alpha-1-Proteinase Inhibitor (Human) with the proposed trade name, "Glassia<sup>TM</sup>", (Kamada-API or AAT). Kamada-API is indicated for chronic augmentation and maintenance therapy of individuals with congenital deficiency of alpha-1-proteinase inhibitor and clinical evidence of emphysema.

Ewa Marszal, Ph.D., of LPD/DH/OBRR, HFM-345 is the chair of this BLA submission. My CMC review is limited only to sterility, pyrogen, bacterial endotoxin, and General Safety testing.

## **Supplement Review Summary**

The Kamada-API drug product (DP) is a sterile liquid solution for intravenous administration, containing 2% Alpha-1-Proteinase Inhibitor (1 g / 50 mL vial), which is prepared from -----(b)(4)----- . The -(b)(4)- are derived from either US Source or recovered plasma and are supplied by -----(b)(4)----- . The composition of the drug substance (DS) and DP is very similar. The only difference between them is that in formulation of the DP, the -----(b)(4)----- with -(b)(4)- Sodium Phosphate, pH - (b)(4)- containing -(b)(4)- NaCl buffer, and then -----(b)(4)----- . In the end, the -----(b)(4)----- .

## **Sterility, Pyrogen, Bacterial Endotoxin, and General Safety Testing**

A. Documents pertaining to these tests that were submitted and reviewed – see Appendix

### **B. Analytical Procedures and Method Validations**

The analytical methods for testing sterility, pyrogen, bacterial endotoxin and General Safety are performed according to the FDA requirements (relevant CFR sections and USP chapters).

#### **1. Sterility Testing**

**Analytical Procedure:** Kamada uses a membrane filtration method for sterility testing based on the -(b)(4)- ----- and the -----(b)(4)----- . To perform this test, they use the -----(b)(4)----- . Both bulk and final container are tested for sterility.

----- (b)(4) -----  
-----  
-----  
-----  
-----  
-----  
-----

----- (b)(4) -----  
-----  
-----  
-----  
-----  
-----  
-----

(b)(4)

## 2. Pyrogen Testing

**Analytical Procedure:** -----(b)(4)-----, an approved contract laboratory, performs the rabbit pyrogenicity test of the DP samples for batch release and stability testing, according to ----(b)(4)----

(b)(4)

### 3. Bacterial Endotoxin Testing

**Analytical Procedure:** For bacterial endotoxin testing, Kamada uses -----(b)(4)-----

Their Bacterial Endotoxin Specification for DP using the -(b)(4)- method is “------(b)(4)-----” (DP is diluted during formulation). Kamada also said that this DP specification limit was calculated based on the recommended dose of Kamada-API: 60 mg active API/ kg/ week. Their package insert instructs to calculate the dose based on the lower limit of the declared label concentration of 2% active API (i.e., 16 mg/mL).

(b)(4)

(b)(4)

(b)(4)



Each analyst is qualified according to the Kamada training and certification SOP. The training of the analyst includes reading the relevant SOP, the training sessions accompanied by a certified analyst. Until officially certified and approved by his superior, an uncertified analyst is not permitted to perform the test without being accompanied by a certified analyst.

----- (b)(4) -----

-----

-----

-----

-----

-----

-----

----- (b)(4) -----

-----

----- (b)(4) -----

-----

-----

-----

-----

-----

-----

-----

----- (b)(4) -----

----- (b)(4) -----

-----

-----

-----

-----

----- (b)(4) -----

----- (b)(4) -----

-----

-----

-----

-----

-----

-----

-----

**27. For bacterial endotoxin testing, please also provide the information requested below:**

- a. Depending on which endotoxin method you choose for lot release testing, please provide the English translation of the SOP for performing this method.**

Kamada Response: As detailed in the answer to Q26, the endotoxin method chosen for lot release testing is the ----- (b)(4) ----- method. The translation of the method SOP, No. N-1P-001-49, is provided in BLA Amendment 12.

- b. Please specify which reference endotoxin standard you are using.**

Kamada Response: The reference endotoxin standard used by Kamada is the following: -----(b)(4)-----  
-----, manufactured by -----(b)(4)-----.

**c. Please specify in the method SOP the sample volumes you use for testing.**

Kamada Response: The Kamada-API sample is diluted at least -----(b)(4)----- prior to testing. The sample volume used for testing is -(b)(4)- of the diluted Kamada-API sample (specified in method SOP, N-1P-001-49, Section 7.7).

**d. Please cite the source(s) of your -----(b)(4)----- reagents in your method SOP and validation SOP.**

Kamada Response: The source of Kamada's -----(b)(4)----- reagents is as follows: -----(b)(4)-----  
----- Test, manufactured by -----(b)(4)-----.  
The method and validation SOPs (N-1P-001-49 and N-1P-001-48, respectively) include the Kamada Catalog No. of the -----(b)(4)----- reagents. The Kamada Catalog No. appears in the validated -(b)(4)-  
----- system that includes the detailed information provided above.

**e. Please provide a Certificate of Quality from the -(b)(4)- reagent supplier that indicates the specific -(b)(4)- correlation of each -(b)(4)- reagent lot.**

Kamada Response: Five Certificates of Analysis indicating the specific RSE/CSE correlation of each -(b)(4)- reagent lot used for the bacterial endotoxin testing according to the ---(b)(4)--- method were provided in Attachment 27-1 (BLA Amendment 12).

**f. Depending on which endotoxin method you choose for lot release testing, please revise your bacterial endotoxin specification accordingly such that it is method-specific.**

Kamada Response: The bacterial endotoxin specification for the -----(b)(4)----- method is “----(b)(4)---  
-----”.

**28. For sterility testing, only the final container (drug product) is tested. 21 CFR 610.12 requires that both the bulk and the final container should be tested. Please provide the following information:**

**a. Please refer to the requirements in 21 CFR 610.12 and modify your method SOP for sterility testing accordingly. Please submit the revised version (English translation).**

Kamada Response: As per 21 CFR 610.12, the sterility testing will be performed on the bulk material in addition to the testing that is already performed on the final container material. The English translations of the method SOP for sterility testing of the final container material, N-1P-0001-23, and the SOP for sterility testing of the Kamada-API bulk material, N-1P-5182-20, were provided in BLA Amendment 12.

**b. Please specify in the method SOP the sample volumes you will use for testing the bulk and the final container.**

Kamada Response: According to the method SOP for the final container material, the sample volume is detailed in the relevant sampling procedure. The translation of the relevant section from the sampling procedure No. P-5182/800 version 14 (pages 5 and 6) is provided in Attachment 28-1 (BLA Amendment 12).

------(b)(4)-----  
-----  
-----  
-----  
-----  
-----

*In the bulk sterility test SOP, N-1P-5182-20, Kamada states that “------(b)(4)----- tested; -(b)(4)- for each growth medium”.*

**c. Please set the sterility specification for the bulk.**

Kamada Response: The sterility specification for the bulk material is the same as that set for the final container material, “Sterile”.

**d. Please provide the evidence that verifies or demonstrates the suitability of the revised method under actual conditions of use (e.g., 14 days of observation).**

Kamada Response: The testing method of the bulk material is Membrane Filtration, and it is performed as per the SOP of the of the final container material, therefore Kamada believes that an additional validation is not required. The sterility validation is described in the original BLA STN 125325/0 Chapter 3.2.P.5.3 Section 1.12.

**29. For pyrogen testing, please provide the following information:**

**a. the method SOP for performing rabbit pyrogen testing.**

Kamada Response: The pyrogen test is designed to assess the risk of a febrile reaction to the product by measuring the rise in temperature of rabbits following intravenous injection of the product. It is performed in accordance with ------(b)(4)-----, an approved contract laboratory. The translated SOP of ------(b)(4)----- is provided in Attachment 29-1 -(b)(4)-Procedure No. PRO/036-H/12 (BLA Amendment 12).

**b. the sample volume used for testing**

Kamada Response: The sample volume used for pyrogen testing is 4 mL of product per kg of rabbit body weight. The sample volume complies with the requirements stated in the 21 CFR 610.13 paragraph (b)(1), the ----(b)(4)--- , and the -----(b)(4)---- Human Alpha-1-Proteinase monograph. In addition to these, the Kamada specification for Active API content is ------(b)(4)----- . The sample volume was calculated according to 16 mg/mL, which represents the worst case of active API content, therefore 60 mg divided by 16 mg/mL results in 3.75 mL, which was rounded to 4 mL.

**c. the evidence that verifies or demonstrates the suitability of the method under actual conditions of use**

Kamada Response: A statement regarding the qualification process of the pyrogen test method, signed by the Quality Assurance Manager at ------(b)(4)----- is provided in Attachment 29-2 (BLA Amendment 12). Briefly, qualification of the pyrogen test method using laboratory animals under actual conditions of use is not possible. This is due to the fact that the Israeli Animal Welfare Law of 1994 (Animal Experimentation Law) and the ethics committee that approves the use of animals for laboratory testing do not permit deliberate injection of animals with pyrogenic material in order to show that a pyrogenic response is obtained in the animals. Therefore, the qualification of the pyrogen test method is limited to using calibrated equipment, maintenance of animals in a controlled environment, training of technicians that perform the test and upholding of GLP.

**30. Please provide the method SOP for performing the General Safety Test. Please specify the sample volumes that are being used for testing.**

Kamada Response: The method SOP for performing the test is provided in Attachment 30-1- -----(b)(4)---- SOP No. 029 (BLA Amendment 12). As stated in the method SOP, the sample volumes that are being used for testing Kamada-API are 0.5 mL per mouse and 5.0 mL per guinea pig as required by the 21 CFR 610.11.

## **APPENDIX**

### **Supporting documents submitted in the Original BLA that were reviewed:**

1. TR-N-1016-06: Validation of Sterility Determination (version 6, translation approved 31-MAR-09)
2. Rep-VL-07073-AM: Validation of AAT Sterility Test Procedure (N-1016)(version 1, effective 10-JUL-07)
3. TR-N-2011-04: Validation of -(b)(4)- Test (version 4, translation approved 8-APR-09)
4. TR-N-2011-05: Validation of -(b)(4)- Test (version 5, effective 1-JUL-07, translation approved 8-APR-09) –  
-(b)(4)- *method*
5. TR-N-1P-0001-48-01: Validation of Testing Endotoxin Concentration in -----(b)(4)----- Method by the  
----- (b)(4)----- (version 1, translation approved 20-APR-09)
6. Rep-VL-07076-AM: Validation of the -(b)(4)- Method for Measuring Bacterial Endotoxins in -----(b)(4)-----  
----- (version 1, effective 27-JAN-08)
7. (Protocol) Addendum Rep-VL-07076-AM/A1: Validation of the -(b)(4)- Method for Measuring Bacterial  
Endotoxins in -----(b)(4)----- (version 1, effective 1-JUL-07)
8. Addendum Rep-VL-07076-AM/A1: Validation of the -(b)(4)- Method for Measuring Bacterial Endotoxins in  
----- (b)(4)----- (version 1, effective 27-JAN-08)
9. Rep-VL-0307-AM/9: Validation of the -(b)(4)- Method for Measuring Bacterial Endotoxins in AAT Drug  
Product Samples (version 1, effective 15-APR-08)
10. Rep-VL-100041-AM: Validation of the -----(b)(4)----- Method for Measuring Bacterial Endotoxins in  
----- (b)(4)----- (version 2, effective 8-APR-09)
11. Rep-VL-100025-AM: Validation of the -----(b)(4)----- Method for Measuring Bacterial Endotoxins in  
AAT Drug Product (version 3, effective 8-APR-09)

### **General Overviews/Summaries:**

1. Section 3.2.S.4.1 Specifications (DS)
2. Section 3.2.S.4.2 Analytical Procedures (DS)
3. Section 3.2.S.4.3 Validation of Analytical Procedures (DS)
4. Section 3.2.S.4.4 Batch Analyses (DS)
5. Section 3.2.S.4.5 Justification of Specifications (DS)
6. Section 3.2.P.5.1 Specifications (DP)
7. Section 3.2.P.5.2 Analytical Procedures (DP)
8. Section 3.2.P.5.3 Validation of Analytical Procedures (DP)
9. Section 3.2.P.5.4 Batch Analyses (DP)
10. Section 3.2.P.5.6 Justification of Specifications (DP)

### **Additional supporting documents submitted in the BLA Amendment 12 that were reviewed:**

1. File # 727009112200.plt: Verification of the Sensitivity of a -(b)(4)- Reagent lot vs. a Endotoxin lot (performed  
and reviewed on 22-NOV-09) (Attachment 26-1)
2. Five Certificates of Analysis for ----(b)(4)--- Control Standard Endotoxin lots (from -----(b)(4)-----,  
dated 27-FEB-08 to 24-JUN-09) (Attachment 27-1)
3. Document P-5182/800 version 14: List of Sample and Reporting of QC Laboratory Results- AAT 2%, 50 mL  
(pages 5-6 showing the sampling procedure for Sterility Testing of bulk and product vials) (Attachment 28-1)
4. TR--(b)(4)- PRO/036-H/12: Pyrogen Test in Rabbits ----- (b)(4)-----  
effective 11-NOV-09, translation approved 4-JAN-10)(Attachment 29-1)
5. -(b)(4)- Quality Assurance Statement (dated 24-DEC-09)(Attachment 29-2)
6. ----(b)(4)--- SOP No. 029: General Safety Test (version 1, approved 28-DEC-09)(Attachment 30-1)
7. TR-N-1P-0001-23-04: Sterility Testing Site and Sterility Tests- General (version 4, translation approved 4-  
JAN-10)
8. TR-N-1-1P-0001-49-02: Bacterial Endotoxin Concentration Test by -----(b)(4)----- Technique (version  
2, translation approved 29-DEC-09)

9. SOP N-1P-5182: Bulk Sterility Test, Kamada-API (version 1, effective 30-DEC-09)