



DBSQC/OCBQ ANALYTICAL METHOD REVIEW MEMO

To: The file: STN 125868/0

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Applicant: Orca Biotherapeutics Inc. (orcabio)

Subject: Review of Sterility, Bioburden, and Endotoxin Analytical Methods used for
TREGZI ((b) (4))

Recommendation: Approval

Executive Summary:

The sterility, bioburden, and endotoxin analytical methods used for testing and release of TREGZI ((b) (4)) and the associated analytic method qualifications, were reviewed. The assays were adequately described and shown to be suitable for their intended purpose.

Conclusion: The analytical methods and their qualifications reviewed for TREGZI ((b) (4)) drug substance and drug product were found to be adequate for their intended use.

Documents Reviewed

Information in sections of the original submission that describe control of Drug Substance (DS) and Drug Product (DP) (3.2.S.4 and 3.2.P.5, respectively), including descriptions of DS and DP specifications, analytical procedures of DS and DP and qualifications of these analytical procedures were reviewed. In addition, the responses to CBER's Information Requests (IRs) received on November 5, 2025 (Amendment #15), November 26, 2025 (Amendment #20), January 16, 2026 (Amendment # 34), February 6, 2026 (Amendment # 38), and March 13, 2026 (Amendment # 45), were also reviewed.

Background:

Orca Biotherapeutics Inc. submitted BLA 125868/0 on August 5, 2025, for TREGZI ((b) (4)) an allogeneic stem cell, and T-cell immunotherapy derived from an HLA-matched donor. TREGZI final drug product (DP) consists of purified cellular components, including hematopoietic stem and progenitor cells (HSPCs), regulatory T cells (Tregs), conventional T cells (Tcons), and a Tcon diluent.

TREGZI is delivered as a set of four distinct drug products that are administered through a carefully orchestrated treatment protocol. Two components (i.e., the HSPC and Treg drug products) are formulated in approximately 100 mL volumes each and shipped fresh for immediate intravenous administration on day 0 of treatment. The conventional T cell component is formulated in a smaller 15 mL volume and undergoes cryopreservation for shipment. This Tcon product is subsequently thawed and diluted with the Tcon diluent between days +2 to +3 at the transplant center before being administered intravenously. This multi-component, staged administration approach reflects the complex nature of this personalized allogeneic immunotherapy designed to harness the therapeutic potential of multiple cell types in treating patients.

This review focuses on the qualification of the sterility, bioburden and endotoxin tests as performed on the DP, to indicate if the product matrix is suitable for testing using the intended test method.

1. Sterility Test DPIntroduction

This test is performed on DP at Orca Biosystems Inc. site in Sacramento, CA. Specification of 'No Growth' must be met for lot release.

Review of Method

The ((b) (4)) sterility test is used for various liquids, solids, and devices in accordance with ((b) (4)). Test samples are ((b) (4)).

The method is described in more detail below together with the tests that were performed to determine suitability of the test method.

Due to the nature of the DP, results from the sterility test will not be available at the time of release. Therefore, Orcabio intends to provide sterility assurance through analysis using a ((b) (4)) test. The methods are described in more detail below together with the tests performed to determine the suitability of the test methods for their intended use.

The original validation reports for sterility lacked sufficient information to complete the review. Therefore, IRs were sent requesting data and clarification to fulfill these

deficiencies. Responses were received on November 5, 2025 (Amendment #15), November 26, 2025 (Amendment #20), January 16, 2026 (Amendment # 34), and March 13, 2026 (Amendment # 45), which were found acceptable and explained below.

(b) (4) Method

Orcabio performed a qualification study of (b) (4) method to demonstrate the method is suitable under the actual conditions of use for the four DPs. The test was performed using (b) (4)

Sterility Test Validation for (b) (4)

Due to the unique nature of cellular and tissue products, there are challenges associated with accruing enough material for subsequent testing. DBSQC is aware strict adherence to testing standards for such products may not always apply due to limited production lot size. The main concern regarding smaller sample test volume for sterility testing is an increased potential for inadvertent infection associated with injection of contaminated product that may be detectable using larger sterility test sample volumes. Therefore, to support the amount of DP used during routine testing (i.e., (b) (4) of each DP), Orcabio added an additional sterility test using a (b) (4)

Passing results from both sterility tests will be needed for release of the batch.

One page has been determined to be not releasable: (b)(4)

(b) (4)

Conclusion

The method suitability test for sterility was performed in accordance with (b) (4) and the test results indicate there is no product inhibition of microorganism growth. Verification of the (b) (4) method was performed and compliant with (b) (4) for microbial identification; however, the demonstrated limit of detection was not appropriate to provide sterility assurance.

2. Bioburden Test Tcon Diluent

Introduction

This test is performed on the Tcon Diluent (i.e., TconD) at Orca Biosystems Inc. site in Sacramento, CA. Specification of (b) (4) for both total aerobic microbial count (TAMC) and total yeast and mold count (TYMC) must be met for release of TconD.

Review of Method

The (b) (4) bioburden test is used to measure microbial contamination levels. Test samples are (b) (4)

The method is described in more detail below together with the tests performed to determine the suitability of the test method for its intended use.

The original submission did not contain a qualification report for bioburden method suitability and lacked sufficient information to complete the review. Therefore, IR was sent requesting the report and additional data to fulfill the deficiency. The response was received on February 6, 2026 (Amendment #38), which was found acceptable and explained below.

Bioburden Test Qualification

Orcabio qualified their (b) (4) bioburden method by performing B&F qualification studies to determine if the method is suitable under the actual conditions of

use for TconD. The test was performed using (b) (4) indicator microorganisms (i.e., (b) (4) on (b) (4) PPQ lots (i.e., (b) (4)) of TconD.

The test for each microorganism was performed using (b) (4)

Conclusion

The method suitability test was performed and compliant with (b) (4), and the test results indicate there is not product inhibition of microbial growth, thus indicating the (b) (4) bioburden test method is appropriate under the actual conditions of use.

3. Endotoxin Test on DP

Introduction

This test is performed on DP at Orca Biosystems Inc. site in Sacramento, CA. Specifications of (b) (4) and (b) (4) must be met for lot release of DPs (i.e., HSPC DP, Treg DP, Tcon Diluent) and Tcon DP, respectively.

Review of Method

(b) (4) -BET is performed to detect or quantitate bacterial endotoxins that may be present in the test sample. (b) (4)

(b) (4)

Method Qualification for DP

Orcabio qualified the DP for the (b) (4)-BET method, to demonstrate that the method is suitable under the actual conditions of use in accordance with (b) (4). The (b) (4)-BET test was qualified by testing (b) (4) conformance batches (i.e., (b) (4) (b) (4)) of DP (i.e., HSPC DP, Treg DP, Tcon DP, and Tcon Diluent).

The (b) (4) for DPs (i.e., HSPC DP, Treg DP, and Tcon Diluent) was calculated to be (b) (4) by (b) (4)

whereas the (b) (4) for Tcon DP was calculated to be (b) (4) by (b) (4)

A suitable testing (b) (4) was determined by performing (b) (4) tests where samples were tested at (b) (4). All sample results showed acceptable (b) (4)

(b) (4) for samples (i.e., HSPC DP, Treg DP, and Tcon Diluent) and at (b) (4) for Tcon DP respectively (acceptance criterion is (b) (4)). Based on the results, (b) (4) testing (b) (4) was selected for release testing for DPs (i.e., HSPC DP, Treg DP, and Tcon Diluent), and (b) (4) for Tcon Diluent, which CBER finds acceptable.

Conclusion

Orcabio submitted bacterial endotoxin concentration results from DPs (i.e., HSPC DP, Treg DP, and Tcon Diluent) and Tcon DP lots, and all were within their proposed release specification (i.e., (b) (4) and (b) (4)). After review of the (b) (4)-BET test, this reviewer concludes the test method was performed and compliant with (b) (4).