# TITLE PAGE

TOXIKON TEST PROTOCOL

FDA GMP REGULATIONS

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**STERILITY ASSAY BY DIRECT TRANSFER OR**

**MEMBRANE FILTRATION METHOD − AAMI**

TOXIKON PROTOCOL NUMBER: PN

*21 CFR Part 820 Compliance*

*Good Manufacturing Practice*

MANAGEMENT OF THE STUDY

Performing Laboratory Sponsor

Toxikon Corporation SponsorName

15 Wiggins Avenue Address

Bedford, MA 01730 City, State ZipCode, Country

# PROTOCOL SIGNATURES

PRINT NAME

Sponsor’s Representative Approval Date

SponsorName2

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PRINT NAME

Quality Assurance Review Date

Toxikon Corporation

15 Wiggins Avenue

Bedford, MA 01730

PRINT NAME

Study Director Signature Date

Toxikon Corporation

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# PURPOSE

The purpose of this study is to determine whether the test article complies with the requirements for sterility, using either the direct transfer or membrane filtration method (method must be specified on the Test Request Form). The membrane filtration method is designed for the passage of a liquid test article through a 0.45 µm membrane filter.

# REFERENCES

This study will be based upon the following references:

## ANSI/AAMI/ISO 11737−2, 2009/(R)2014 − Sterilization of Medical Devices − Microbiological Methods − Part 2: Tests of Sterility Performed in the Definition, Validation and Maintenance of a Sterilization Process.

## ANSI/AAMI/ISO 11737−1, 2006/(R)2011 − Sterilization of Health Care Products − Microbiological Methods − Part 1: Determination of the Population of Microorganisms on Product.

## ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

# COMPLIANCE

This study conforms to the current 21 CFR Part 820 Good Manufacturing Practices, as applicable to a testing laboratory. Toxikon is accredited to ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories. Toxikon’s Quality System also encompasses the general principles and practices of GxP regulations, specifically GLPs.

# IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor will supply the following information on a GMP Test Requisition Form or other correspondence, wherever applicable (excluding confidential or trade secret information).

## Test Article:

Name: To Be Determined (TBD)

CAS/Code Number: TBD

Lot/Batch Number: TBD

## Control Article(s) (Toxikon Supplied, unless specified by the Sponsor):

# IDENTIFICATION OF TEST SYSTEM

## Direct Transfer:

As recommended in AAMI, the test system is direct transfer of the test article into appropriate media.

## Membrane Filtration:

The test system is a membrane filtration unit that facilitates the aseptic handling of the test article and allows the processed membrane to be removed aseptically for inoculation of appropriate media.

# JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

If using the direct transfer method, the test article will be administered, *in vitro*, directly to the test system. If the membrane filtration method is selected, the test article will first be extracted in an appropriate vehicle, the extract will be membrane filtered, and the filters will be administered *in vitro* to the test system.

# EXPERIMENTAL DESIGN AND DOSAGE

## Test Article:

### Direct Transfer:

#### The test article will be prepared per AAMI requirements. Solids or devices will be aseptically transferred to liquid media. Liquids miscible with aqueous vehicles will be aseptically transferred to liquid media. Other test article preparation will be as directed by the Sponsor. The quantity of test article assayed will be as specified by AAMI, unless otherwise specified by the Sponsor.

#### Inoculation:

The test article will be aseptically and directly transferred to the volume of Trypticase Soy Broth (TSB), as specified by AAMI.

### Membrane Filtration:

#### The test article will be prepared per AAMI requirements. Liquids miscible with aqueous vehicles will aseptically be transferred to the membrane filter. Filtration rate may be increased by the addition of a diluting fluid. Solutions or suspensions may be prepared for filterable solid test articles. Fluid D will be passed through the sterile pathways of devices. Other test article preparation will be as directed by the Sponsor. The quantity of test article assayed will be as specified by AAMI guidelines.

#### Filtration:

The test article will be passed through a 0.45 µm membrane filter. Following filtration, the membrane may be washed with three 100 mL volumes of sterile Phosphate Buffer Solution (PBS).

Upon completion of filtration, the filter will be immersed in an appropriate volume of TSB.

## Incubation:

### Direct Transfer:

The TSB vessels will be incubated at 30 ± 2 °C for fourteen days. At intervals during the incubation period and at its conclusion, the media will be examined for macroscopic evidence of microbial growth.

### Membrane Filtration:

The TSB vessels will be incubated at 30 ± 2 °C for fourteen days. Each specimen will be evaluated for sterility during the fourteen day period.

## If the product being tested renders the medium turbid, so that the presence or absence of microbial growth cannot be determined by visual examination, 14 days after the beginning of incubation, portions (each not less than 1 mL) of the turbid medium will be transferred to fresh vessels of the same medium. The original and transfer vessels will be incubated for not less than 4 additional days.

## Controls:

### Direct Transfer:

#### Negative Controls (Sterility Assurance):

The sterility of the growth media will be verified where appropriate. Growth must not be observed for the negative controls.

#### Positive Controls (Growth Promotion Verification):

Growth promotion characteristics will be verified for TSB per AAMI. Growth must be observed for the positive controls.

### Membrane Filtration:

#### Negative Control Articles (Sterility Assurance):

The sterility of the growth media and extraction fluid will be verified where appropriate. Growth must not be observed for the negative control articles.

#### Positive Control Articles (Growth Promotion Verification):

Growth promotion characteristics will be verified for TSB per AAMI. Growth must be observed for the positive control articles.

# EVALUATION CRITERIA

## The positive and negative controls are utilized to verify the proper functioning of the test system. The test article meets the requirements for Sterility if it fails to demonstrate microbial contamination upon visual examination. No growth should be observed for the test article.

## Control of Bias Statement:

The study as designed employs methodology to minimize uncertainty of measurement and control of bias for data collection and analysis, which includes but is not limited to:  control data (retrospective, concurrent, or prospective), system suitability assessment, randomization, method controls such as blanks and replicates, or others as required by the specific study or guideline.  Methods employed will be specified in the final report.

# RECORDS

## Original raw data will be archived by Toxikon Corporation.

## A copy of the final report and any report amendments will be archived by Toxikon Corporation.

## The original final report and a copy of any protocol amendments or deviations will be forwarded to the Sponsor.

## All used and unused test article will be handled as specified on the GMP Test Requisition Form. If not indicated on the GMP Test Requisition Form, all remaining test article will be disposed.

## Test article retention upon study completion is the responsibility of the Sponsor.

# CONFIDENTIALITY AGREEMENT

Per corporate policy, confidentiality shall be maintained in general, and in specific accordance with any relevant agreement specifically executed between Toxikon and the Sponsor.

# UNFORESEEN CIRCUMSTANCES

All unforeseen circumstances will be documented in the raw data. Any unforeseencircumstances that affect the integrity of the study will be discussed in the final report.

# PROTOCOL AMENDMENTS/DEVIATIONS

All changes to the approved protocol and the reason for the changes will be documented in writing, signed by the Study Director, dated, and maintained with the protocol. A Protocol Amendment/Deviation Report (PADR) will be generated as closely as possible to the time of the change. The document will be created and signed by the Study Director and sent to the Sponsor. Sponsor’s signature will be required for amendments to indicate approval of the amendment. Acknowledgement of notification of deviations will either be with a signature or other form of documentation.

# APPENDIX I: Software Systems

The following are the proposed software systems to be used during the conduct of this study. The actual systems used, as well as 21 CFR Part 11 compliance if applicable, will be documented in the final report.

|  |  |  |  |
| --- | --- | --- | --- |
| **Software** | **Use** | **Publisher/Vendor** | **Location** |
| Adobe Acrobat 8, 9, and 10 Professional | Document preparation | Adobe Systems, Inc. | San José, CA |
| Matrix Gemini 5.3.5 | Laboratory Information Management System | Autoscribe Limited | Reading, UK |
| MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite | Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools) | Microsoft Corporation | Redmond, WA |
| Rees Scientific Centron  Presidio 3.0 | Automated Environmental Monitoring | Rees Scientific | Trenton, NJ |
| Report Automation 1.0 | Custom software (add-in) for final report generation, review, approval, distribution to sponsors, and storage | Court Square Group | Springfield, MA |
| TMS Web 7 | Document management for SOPs and training records management software system | Quality Systems Integrators | Eagle, PA |
| Toxikon Protocol Manager 1.0 | Protocol requisition application | Custom developed | Toxikon Corporation, Bedford, MA |