FINAL GMP REPORT: 21-00512-M2

AMOEBOCYTE LYSATE TEST FOR DETECTION AND QUANTITATION OF ENDOTOXINS (KINETIC–QCL METHOD) – USP

Test Article

Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021

21 CFR Part 820 Compliance
Good Manufacturing Practice

Report Date

5/3/2021

Study Director

Linda Haggerty, M.S.

Sponsor

Malema Sensors
1060 S. Rogers Circle
Boca Raton, FL 33487

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STUDY SUMMARY

One (1) test article of Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021, contained within a flow cell, was analyzed for endotoxin using the chromogenic method - USP <85>, Bacterial Endotoxins Test. The flow cell fluid pathway was filled with 230.0 mL of Sterile Water for Injection (SWFI) heated to 37 ± 1 ºC; the SWFI remained in contact with the fluid pathway at room temperature for 60 ± 2 minutes. The pH of the extract was 6.26 and no pH adjustment was required. The extract was assayed in duplicate at the neat concentration. A Positive Product Control (PPC) was prepared containing 0.09 mL of the test article extract and 0.01 mL of the 5 EU/mL endotoxin standard to give a final concentration of 0.5 EU/mL. Water for Bacterial Endotoxin Test (BET) and SWFI were used as the negative controls. The Positive Product Control and the negative controls verified proper functioning of the test system. A standard curve was prepared with *Escherichia coli* (*E. coli*) endotoxin at concentrations ranging from 5.0 to 0.005 EU/mL. The absolute value of correlation coefficient for the linear regression was calculated to be 0.995. The test article extract contained < 0.0500 EU/mL and < 11.5 EU/Device of bacterial endotoxin at the neat concentration. The test article extract was within the limit of 20 EU/Device and meets the requirements of USP<85> Bacterial Endotoxins Test, current revision. Refer to Toxikon study number 21-00512-M1 for Validation.

STUDY DIRECTOR AND QUALITY ASSURANCE SIGNATURES AND VERIFICATION DATES

SIGNATURES

Signature Information	
Protocol Number	p21-0202-00a
Study Director	Linda Haggerty, M.S.
Company	Toxikon Corporation

VERIFICATION DATES

The study initiation day is the date the protocol is signed by the Study Director.

Verification Dates	
Test Article Receipt	2/10/2021
Project Log	4/26/2021
Study Initiation	4/26/2021
Study Completion	5/3/2021

J. M. Nigrelli
James Nigrelli, B.S.
Quality Assurance

5/3/21
Date

Linda Haggerty
Linda Haggerty, M.S.
Study Director

5-3-2021
Date

1.0 PURPOSE

The purpose of this study was to determine the concentration of bacterial endotoxins present in or on the test article. The test system used Amoebocyte Lysate obtained from aqueous extracts of the circulating amoebocytes of the horseshoe crab, *Limulus polyphemus*. Amoebocyte Lysate, prepared and characterized for use, is commercially available and reacts with a colorimetric substrate to detect endotoxin.

When used according to USP <85> Bacterial Endotoxins Test, it is substituted for the USP Rabbit Pyrogen test for the end–product testing of human injectable drugs (including biological products), animal injectable drugs, and medical devices. The USP Bacterial Endotoxins Test is the official test referenced in specific USP monographs. The concentration was determined using a standard curve of endotoxin of a reference endotoxin, with the quantities of endotoxin being expressed in defined Endotoxin Units (EU)/mL.

2.0 REFERENCES

The study was based upon the following references:

- United States Pharmacopeia 43, National Formulary 38, 2020. <85> Bacterial Endotoxins Test.
- United States Pharmacopeia 43, National Formulary 38, 2020. <161> Medical Devices – Bacterial Endotoxin and Pyrogen Tests.
- Limulus Amoebocyte Lysate (LAL), Kinetic-QCL™, Kit Instruction, Lonza.
- ISO/IEC 17025, 2017, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

This study conformed to the current 21 CFR Part 820 Good Manufacturing Practice, as applicable to a testing laboratory. Toxikon is accredited to ISO/IEC 17025, 2017, 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.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

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

4.1 Test Article:

Name: Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021

CAS/Code Number: Not Supplied by Sponsor (N/S)

Lot/Batch Number: N/S

Physical State: N/S

Color: N/S

Expiration Date: N/S

Density: N/S

Stability: N/S

Sterility: Sterile

Sterilization Conditions: N/S

Storage Condition: Room Temperature

Safety Precautions: N/S

Intended Use: N/S

4.2 Negative Control Articles (Toxikon Supplied):

4.2.1 Negative Control Article 1:

Name: USP Sterile Water for Injection (SWFI)

Toxikon QC Number: CSC-20-11-00126

4.2.2 Negative Control Article 2:

Name: Water for Bacterial Endotoxins Test (BET)

Toxikon QC Number: CSC-20-07-00098

4.3 Positive Control Articles (Toxikon Supplied):

4.3.1 Positive Control Article 1:

Name: *Escherichia coli* endotoxin

Toxikon QC Number: CSC-20-10-00034

4.3.2 Positive Control Article 2:

Name: Limulus Amoebocyte Lysate

Toxikon QC Number: CSC-20-10-00033

5.0 IDENTIFICATION OF TEST SYSTEM

The Bacterial Endotoxins Test, Kinetic Chromogenic technique estimated the concentration of bacterial endotoxins present in or on the test article. The test system used Amoebocyte Lysate obtained from aqueous extracts of the circulating amoebocytes of the horseshoe crab, *Limulus polyphemus*. Amoebocyte Lysate, prepared and characterized for use, is commercially available and reacts with a colorimetric substrate to detect endotoxin.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

The Bacterial Endotoxins Test was recommended by USP guidelines as a method to estimate the concentration of bacterial endotoxins present in or on the test article.

6.2 Route of Administration:

Each test article extract was spiked, *in vitro*, directly with a known amount of endotoxin.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Test Article Preparation:

7.1.1 Liquid Test Articles:

All test articles were collected in endotoxin–free containers and were stored per Sponsor-specified storage conditions until tested. If conditions are not specified, liquid samples were stored at room temperature. No preparation was required, unless specified by the Sponsor.

7.1.2 Preparation of Devices (Test Articles) and Dosage:

The fluid pathway of the Test Article was filled with 230.0 mL of SWFI heated to 37 ± 1 ºC. The SWFI remained in contact with the fluid pathway at room temperature for 60 ± 2 minutes. The temperature was recorded at the beginning and end of the extraction period.

Note: Toxikon's standard procedure is to use SWFI as the extraction fluid unless specified by the Sponsor.

For unusually large or small devices, the surface area of the device, which comes in contact with the patient, was used as an adjustment factor in selecting the rinsing or extracting volume. The endotoxin limit was adjusted accordingly.

It is necessary for the sample to have a pH in the range of 6.0-8.0. The pH of the extract was found to be pH 6.26 and no pH adjustment was necessary. The pH of the sample was measured using a calibrated pH probe.

7.2 Reagent and Material Preparation:

All reagents were prepared per manufacturer's recommendations.

7.2.1 Preparation of Endotoxin Standard:

The Control Standard Endotoxin (CSE) in the Kinetic–QCL Kit that was previously standardized by the manufacturer against the Reference Standard Endotoxin (RSE) was used. The Endotoxin Standard was reconstituted with the specified amount of Bacterial Endotoxin Water and vortexed for fifteen (15) minutes. The reconstituted endotoxin had a potency of 50 Endotoxin Units (EU)/mL. A previously reconstituted endotoxin standard was vortexed for fifteen (15) minutes and was stored (mouth wrapped with parafilm) at 4 ± 2 ºC for up to four (4) weeks. The endotoxin standard was only used with the reagents in a particular kit. All unused working standard would not be stored for more than one (1) day.

7.2.2 Preparation of Kinetic-QCL LAL Reagent:

The reagent shall not be prepared until immediately prior to use. The Amoebocyte Lysate powder was collected into the bottom of the vial by tapping on a firm surface. The metal seal of the reagent was torn off and the gray stopper gently removed. The powder was reconstituted with the amount of Water for BET specified by the manufacturer. Reconstituted Kinetic–QCL LAL Reagent was stable for eight (8) hours at 2–8 ºC.

7.2.3 Preparation of Glassware and Utensils:

Any glass containers, test tubes, utensils, etc., used in this assay were depyrogenated. The depyrogenation was done by wrapping the utensils in foil and heating for at least thirty (30) minutes at 250 ºC.

7.3 Test Method:

To calculate the concentration of bacterial endotoxins in the test article, the test article extract was tested at the neat concentration in duplicate and compared to a standard curve containing endotoxin concentrations of 5 EU/mL, 0.5 EU/mL, 0.05 EU/mL, and 0.005 EU/mL.

7.3.1 Preparation of Endotoxin Standard Curve:

The endotoxin standard was prepared as specified in Section 7.2.1.

The prepared endotoxin was diluted to a concentration of 5 EU/mL by adding 0.1 mL of the 50 EU/mL endotoxin stock into 0.9 mL of Water for BET and vortexed for one (1) minute. A concentration of 0.5 EU/mL was obtained by transferring 0.1 mL of the 5 EU/mL into 0.9 mL of Water for BET, and vortexed for one (1) minute. A concentration of 0.05 EU/mL was obtained by transferring 0.1 mL of the 0.5 EU/mL into 0.9 mL of Water for BET, and vortexed for one (1) minute. A concentration of 0.005 EU/mL was obtained by transferring 0.1 mL of the 0.05 EU/mL into 0.9 mL of Water for BET, and vortexed for one (1) minute.

7.3.2 Preparation of Positive Product Control (PPC):

The positive product control spike contained 0.5 EU/mL if the Pass/Fail cut off was less than 1.0 EU/mL. If the Pass/Fail cut off was greater than 1.0 EU/mL, the spike was 5.0 EU/mL. The calculated mean amount of endotoxin in the spiked eluate/extract, when referenced to the standard curve, must be within 50–200% to be considered to neither enhance nor inhibit this assay.

To make the PPC, 10 µL of the 5 EU/mL standard was added to 90 µL of the test article extract for a final concentration of 0.5 EU/mL. The actual volume of test article in the appropriate well of the microplate is **always** 0.1 mL. The filled plate was placed in the microplate reader and the lid closed. The plate was pre–incubated for ≥ 10 minutes at 37 ± 1 ºC.

7.3.3 Addition of Lysate:

0.1 mL of reconstituted Amoebocyte Lysate was added to each well of the microplate containing 0.1 mL of the test article or control. Amoebocyte Lysate was added to the negative controls first, and from the lowest to highest concentration in each test series. The microplate was immediately placed in the reader to initiate the test.

7.3.4 Preparation of the Negative Control and Blank:

The blank for the standard curve should always be Water for BET. In the case of a device that was rinsed, immersed, or flushed, a 100 µL aliquot of the vehicle used in the extraction process was assayed as the negative control in duplicate.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Date:

Results were printed out at the completion of the assay. Replicate samples were run in order to establish good technique and low coefficient of variation. The coefficient of variation, CV, equals the “sample” standard deviation of the reaction times divided by the mean and it is usually expressed as a percent. The % CV of the reaction times for replicate of a sample should be less than 10%.

The absolute value of the coefficient of correlation of the calculated standard curve should be ≥ 0.980.

If the PPC value is outside the range of 50-200% of the known spike concentration, the results show either inhibition or enhancement of the assay.

A device that fails this test can be retested once by another Bacterial Endotoxins Test. For devices that cannot be tested by the Bacterial Endotoxins Test because of non-removable inhibition or enhancement, the Pyrogen Test (USP <151>) is applied.

For medical devices, the endotoxin limit is 20.0 EU/Device except for those medical devices in contact with cerebrospinal fluid where the limit is 2.15 EU/Device.

8.2 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: concurrent control data, system suitability assessment, and method controls such as blanks and replicates.

9.0 RESULTS

The test article, Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021, was tested for endotoxin using the chromogenic method – USP <85>, Bacterial Endotoxins Test. The absolute value of the correlation coefficient for the linear regression was calculated to be 0.995. The test article, Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021, contained < 0.0500 EU/mL and < 11.5 EU/Device of bacterial endotoxin at the neat concentration.

10.0 CONCLUSION

The test article, Polyether ether ketone (PEEK) Sensors- (Solvay KT-880 NT)Part# CSEN-8103-C-153I0SH1-001 Serial # 62912-0001 and 62912-0002 . Manufacturing date : Feb 3rd 2021, meets the requirements for the LAL endotoxin test, chromogenic method - USP <85>, Bacterial Endotoxins Test, current revision. Refer to Toxikon study number 21-00512-M1 for Validation. The study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

11.0 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.
- The test articles, used and unused, will be returned to the Sponsor.
- Test article retention upon study completion is the responsibility of the Sponsor.

12.0 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.

13.0 UNFORESEEN CIRCUMSTANCES

All unforeseen circumstances will be documented in the raw data. However, there were no unforeseen circumstances that affected the integrity of the study.

14.0 PROTOCOL AMENDMENTS/DEVIATIONS

There were no protocol amendments or deviations. No changes to the protocol were required.

TABLE 1:
Endotoxin Quantity

Test Article #	Dilution	pH	%CV	EU/mL	EU/Device	Valid PPC (Yes/No)
1	Neat	6.26	1.56	< 0.0500	< 11.5	Yes

EU/mL = Endotoxin Units/milliliter; EU/Device = Endotoxin Units/Device; CV = Coefficient of Variation; PPC = Positive product Control

APPENDIX I:
Software Systems

Software	Use	21 CFR Part 11 Status	Publisher/Vendor	Location
Adobe Acrobat 8, 9, and 10 Professional	Document preparation	Not Applicable	Adobe Systems, Inc.	San José, CA
Matrix Gemini 5.3.19	Laboratory Information Management System	Compliant	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)	Not Applicable	Microsoft Corporation	Redmond, WA
Rees Scientific Centron Presidio 3.0	Automated Environmental Monitoring	Compliant	Rees Scientific	Trenton, NJ
TMS Web 7	Document management for SOPs and training records management software system	Compliant	Quality Systems Integrators	Eagle, PA
Toxikon Protocol Manager 1.0	Protocol requisition application	Not Applicable	Toxikon Corporation	Bedford, MA
Win KQCL Version 3.1	Micro plate reader	Compliance-Ready	Lonza	Walkersville, MD

TOXIKON TEST PROTOCOL
FDA GMP REGULATIONS
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AMOEBOCYTE LYSATE TEST FOR DETECTION AND QUANTITATION OF ENDOTOXINS (KINETIC–QCL METHOD) – USP

TOXIKON PROTOCOL NUMBER: p21-0202-00a

21 CFR Part 820 Compliance
Good Manufacturing Practice

MANAGEMENT OF THE STUDY

Performing Laboratory
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15 Wiggins Avenue
Bedford, MA 01730

Sponsor
Malema Sensors
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Boca Raton, FL 33487

PROTOCOL SIGNATURES

Kalash Shamsh.
PRINT NAME

Shamsh.

02/5/21.
Date

Sponsor's Representative Approval
Malerna Sensors
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Boca Raton, FL 33487

Felice Randi LaMadeleine
PRINT NAME

Felice Randi LaMadeleine

02/08/2021
Date

Quality Assurance Review
Toxikon Corporation
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Bedford, MA 01730

LINDA HAGGERTY
PRINT NAME

Linda Haggerty

4-26-21
Date

Study Director Signature
Toxikon Corporation
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Appendix:

1.0 PURPOSE

The purpose of this study is to determine the concentration of bacterial endotoxins present in or on the test article. The test system will use Amoebocyte Lysate obtained from aqueous extracts of the circulating amoebocytes of the horseshoe crab, *Limulus polyphemus*. Amoebocyte Lysate, prepared and characterized for use, is commercially available and reacts with a colorimetric substrate to detect endotoxin.

When used according to USP <85> Bacterial Endotoxins Test, it may be substituted for the USP Rabbit Pyrogen test for the end-product testing of human injectable drugs (including biological products), animal injectable drugs, and medical devices. The USP Bacterial Endotoxins Test is the official test referenced in specific USP monographs. The concentration is determined using a standard curve of endotoxin of a reference standard endotoxin, with the quantities of endotoxin being expressed in defined Endotoxin Units (EU)/mL.

2.0 REFERENCES

The study will be based upon the following references:

- United States Pharmacopeia 43, National Formulary 38, 2020. <85> Bacterial Endotoxins Test.
- United States Pharmacopeia 43, National Formulary 38, 2020. <161> Medical Devices – Bacterial Endotoxin and Pyrogen Tests.
- Limulus Amoebocyte Lysate (LAL), Kinetic–QCL™, Kit Instruction, Lonza.
- ISO/IEC 17025, 2017, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

This study conforms to the current 21 CFR Part 820 Good Manufacturing Practice, as applicable to a testing laboratory. Toxikon is accredited to ISO/IEC 17025, 2017, 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.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

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

4.1 Test Article:

Name: To Be Determined (TBD)

CAS/Code Number: TBD

Lot/Batch Number: TBD

Physical State: TBD

Color: TBD

Expiration Date: TBD

Density: TBD

Stability: TBD

Sterility: TBD

Sterilization Conditions: TBD

Storage Condition: TBD

Safety Precautions: TBD

Intended Use: TBD

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

4.2.1 Negative Control Article 1:

Name: USP Sterile Water for Injection (SWFI)

Toxikon QC Number: To Be Determined (TBD)

4.2.2 Negative Control Article 2:

Name: Water for Bacterial Endotoxin Test (BET)

Toxikon QC Number: To Be Determined (TBD)

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

Name: *Escherichia coli* endotoxin

Toxikon QC Number: To Be Determined (TBD)

5.0 IDENTIFICATION OF TEST SYSTEM

The Bacterial Endotoxins Test, Kinetic Chromogenic technique will estimate the concentration of bacterial endotoxins present in or on the test article. The test system will use Amoebocyte Lysate obtained from aqueous extracts of the circulating amoebocytes of the horseshoe crab, *Limulus polyphemus*. Amoebocyte Lysate, prepared and characterized for use, is commercially available and reacts with a colorimetric substrate to detect endotoxin.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

6.1 Justification of Test System:

The Bacterial Endotoxins Test is recommended by USP guidelines as a method to estimate the concentration of bacterial endotoxins present in or on the test article.

6.2 Route of Administration:

The liquid test article or the test article extract will be spiked *in vitro* directly with a known amount of endotoxin.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Test Article Preparation:

7.1.1 Liquid Test Articles:

All test articles will be collected in endotoxin–free containers and will be stored per Sponsor-specified storage conditions until tested. If conditions are not specified, liquid samples will be stored at room temperature. No preparation will be required, unless specified by the Sponsor.

7.1.2 Preparation of Devices (Test Articles) and Dosage:

Devices will be stored at room temperature unless other storage conditions are specified by the Sponsor. Rinse or immerse the devices with an appropriate volume of extracting fluid that has been maintained at 37 ± 1 °C or as specified by the Sponsor. The test articles will be extracted for 60 ± 2 minutes at room temperature or at 37 ± 1 °C or as specified by the Sponsor. The temperature will be recorded at the beginning and end of the extraction period. For devices labeled “non-pyrogenic fluid pathway” the fluid pathway will be filled or flushed with an appropriate volume of extracting fluid that will be heated to 37 ± 1 °C.

For devices being flushed, the extraction fluid will be held in the fluid pathway for 60 ± 2 minutes at room temperature or as specified by the Sponsor. The effluents will be pooled, if applicable.

Note: Toxikon’s standard procedure is to use SWFI as the extraction fluid unless specified by the Sponsor.

For unusually large or small devices, the surface area of the device, which comes in contact with the patient, may be used as an adjustment factor in selecting the rinsing or extracting volume. The endotoxin limit will be adjusted accordingly.

It is necessary for the sample to have a pH in the range of 6.0-8.0. If the pH is not in this range, the pH will be adjusted using an endotoxin-free buffer. The pH of aliquots of each sample will be measured using a calibrated pH probe. Endotoxin-free buffer will be added in small amounts until the pH of each aliquot reaches 6.0-8.0, and the ratio of buffer to sample aliquot will be used to determine the amount of buffer to be added to the original sample.

7.2 Reagent and Material Preparation:

Prepare all reagents per manufacturer’s recommendations.

7.2.1 Preparation of Endotoxin Standard:

The Control Standard Endotoxin (CSE) in the Kinetic–QCL Kit that has been previously standardized by the manufacturer against the Reference Standard Endotoxin (RSE) will be used. The endotoxin standard will be reconstituted with the specified amount of Water for BET, and vortexed for 15 minutes.

The reconstituted endotoxin will have a potency of 50 Endotoxin Units (EU)/mL. The reconstituted endotoxin standard will be stored (mouth wrapped with parafilm) at 4 ± 2 °C for up to four weeks. The endotoxin standard will be only used with the reagents in a particular kit. If using previously reconstituted endotoxin, the endotoxin shall be vigorously vortexed for not less than 15 minutes before diluting. All unused working standard will not be stored for more than one (1) day.

7.2.2 Preparation of Kinetic-QCL LAL Reagent:

The reagent shall not be prepared until immediately prior to use. The Amoebocyte Lysate powder will be collected into the bottom of the vial by tapping on a firm surface. The metal seal of the reagent will be torn off and the gray stopper gently removed. The powder will be reconstituted with the amount of Water for BET specified by the manufacturer. Reconstituted Kinetic–QCL LAL Reagent is stable for 8 hours at 2–8 °C; otherwise it can be stored at –10 °C or below for up to two weeks. The reconstituted reagent will only be frozen and thawed once.

7.2.3 Preparation of Glassware and Utensils:

Any glass containers, test tubes, utensils, etc., used in this assay will be depyrogenated. The depyrogenation will be done by wrapping the utensils in foil and heating for at least 30 minutes at 250 °C.

7.3 Test Method:

To calculate the concentration of bacterial endotoxins in the test article, the test article extract or liquid sample will be tested at the neat concentration in duplicate and compared to a standard curve containing endotoxin concentrations of 5 EU/mL, 0.5 EU/mL, 0.05 EU/mL, and 0.005 EU/mL.

7.3.1 Preparation of Endotoxin Standard Curve:

The endotoxin standard will be prepared as specified in Section 7.2.1.

The prepared endotoxin will be diluted to a concentration of 5 EU/mL by adding 0.1 mL of the 50 EU/mL endotoxin stock into 0.9 mL of Water for BET, and vortexing for one minute. A concentration of 0.5 EU/mL will be obtained by transferring 0.1 mL of the 5 EU/mL into 0.9 mL of Water for BET, and vortexing for one minute. A concentration of 0.05 EU/mL will be obtained by transferring 0.1 mL of the 0.5 EU/mL into 0.9 mL of Water for BET, and vortexing for one minute. A concentration of 0.005 EU/mL will be obtained by transferring 0.1 mL of the 0.05 EU/mL into 0.9 mL of Water for BET, and vortexing for one minute.

7.3.2 Preparation of Positive Product Control (PPC):

The Positive Product Control spike will contain 0.5 EU/mL if the Pass/Fail cut off is less than 1.0 EU/mL. If the Pass/Fail cut off is greater than 1.0 EU/mL, the spike will be 5.0 EU/mL. The calculated mean amount of endotoxin in the spiked eluate/extract, when referenced to the standard curve, must be within 50–200% to be considered to neither enhance nor inhibit this assay.

To make the PPC, 10 µL of the 5 EU/mL standard will be added to 90 µL of the test article extract for a final concentration of 0.5 EU/mL. The actual volume of test article in the appropriate well of the microplate is always 0.1 mL. The filled plate will be placed in the microplate reader and the lid closed. The plate will be pre–incubated for ≥ 10 minutes at 37 ± 1 °C.

7.3.3 Addition of Lysate:

0.1 mL of reconstituted Amoebocyte Lysate will be added to each well of the microplate containing 0.1 mL of the test article or control. Amoebocyte Lysate will be added to the

negative controls first, and from the lowest to highest concentration in each test series. The microplate will be immediately placed in the reader to initiate the test.

7.3.4 Preparation of the Negative Control and Blank:

The blank for the standard curve should always be Water for BET. In the case of a device that is rinsed, immersed, or flushed, a 100 µL aliquot of the vehicle used in the extraction process is assayed as the negative control in duplicate.

8.0 EVALUATION CRITERIA

8.1 Evaluation of Data:

Results will be printed out at the completion of the assay. Replicate samples should be run in order to establish good technique and low coefficient of variation. The coefficient of variation, CV, equals the "sample" standard deviation of the reaction times divided by the mean and it's usually expressed as a percent. The % CV of the reaction times for replicate of a sample should be less than 10%.

The absolute value of the coefficient of correlation of the calculated standard curve should be ≥ 0.980.

If the PPC value is outside the range of 50–200% of the known spike concentration, the results show either inhibition or enhancement of the assay.

A device that fails this test can be retested once by another Bacterial Endotoxins Test. For devices that cannot be tested by the Bacterial Endotoxins Test because of non-removable inhibition or enhancement, the Pyrogen Test (USP <151>) is applied.

For medical devices, the endotoxin limit is 20.0 EU/Device except for those medical devices in contact with cerebrospinal fluid where the limit is 2.15 EU/Device.

8.2 Control of Bias Statement:

The study as designed employs methodology to minimize uncertainty of measurement and to control 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.

9.0 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 the protocol and any protocol amendments or deviations will be forwarded to the Sponsor.
- All used and unused test article will be handled as specified on the Test Requisition Form. If not indicated on the Test Requisition Form, all remaining test article will be disposed.
- Test article retention upon study completion is the responsibility of the Sponsor.

10.0 CONFIDENTIALITY AGREEMENT

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

11.0 UNFORESEEN CIRCUMSTANCES

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

12.0 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 (PA) or a Protocol Deviation (PD) 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 (PA) to indicate approval of the amendment. Acknowledgement of notification of deviations is preferred and may 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 5.3.19	Laboratory Information Management System	Autoscribe Limited	Reading, UK
MS Office 2010 Small Business Suite and MS Office 2013 Professional Suite and higher	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	Toxikon Corporation	Bedford, MA
Win KQCL Version 3.1	Micro plate reader	Lonza	Walkersville, MD