TOXIKON

Leaders in Life Science and Technology

TOXIKON FINAL NON–GLP REPORT: 10–0402–N1

MICROBIAL BARRIER PERFORMANCE TESTING OF INTACT PACKAGES – SterilEnz^{R}–II/G, 2G–616–TC–5X5 –

Test Article
SterilEnz–II/G, 2G–616–TC–5X5

Author
Sharon A. Malia, Ph.D.

Final Report Date
March 8, 2010

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
PAW BioScience Products, Inc.
28 Eaton Road
Eatontown, NJ 07724

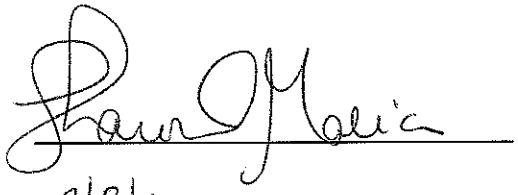
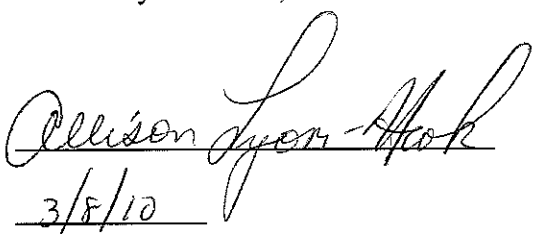
TABLE OF CONTENTS

STUDY SUMMARY

A Microbial Barrier Performance Test was carried out to evaluate the ability of the test article, SterilEnz-11/G, 2G–616–TC–5X5, to effectively serve as a complete microbial barrier, therefore maintaining its sterile environment until it reaches its point of end use. SterilEnz–11/G, 2G–616–TC–5X5 was challenged with the test microorganism *Bacillus subtilis* (ATCC # 6633). The complete outer surface of the sealed pouch containing the connector was challenged by immersing it in a buffer solution containing 1 x 10^5 CFU/mL of the indicator organism *Bacillus subtilis*. Twenty four (24) test articles (TA) and four (4) positive control test articles (PCTA) were immersed for approximately 1 minute and the challenge suspension was allowed to remain on the test article for 4 hours. After the contact period at room temperature, the area of the outer pouch that is most suitable for opening the package was wiped down with a disinfectant. This was repeated three (3) times prior to opening the package. The entire internal surface area of the pouch and the connector were tested for sterility. Trypticase Soy Broth (TSB) was used to fill the pouch and remained in the pouch for 30 minutes. The media was transferred to a sterile flask and incubated at 30–35 °C for 14 days.

The contents of the vessels were examined for macroscopic evidence of microbial growth daily for 14 days. Over this 14 day period, there was no evidence of microbial growth. Therefore, it is concluded that the SterilEnz-11/G, 2G–616–TC–5X5 serves as a complete microbial barrier and its sterile environment will remain sterile following a worst-case microbial challenge.

STUDY DIRECTOR AND QUALITY ASSURANCE SIGNATURES AND VERIFICATION DATES

Protocol Number	P10-0432-00B
Study Director:	Sharon A. Malia, Ph.D.
Company:	Toxikon Corporation
Signature:	
Date:	3/8/10
Study Supervisor:	Sharon A. Malia, Ph.D.
Quality Assurance:	Allison Lyons-Hook, B.A.
Signature:	
Date:	3/8/10

VERIFICATION DATES:
The Study Initiation Date is the date the protocol is signed by the Study Director.

Test Article Receipt:	01/27/10
Project Log Date:	01/28/10
Study Initiation Date:	02/18/10
Technical Initiation:	02/18/10
Technical Completion:	03/04/10

1.0 PURPOSE

The purpose of this study was to evaluate the ability of the test article, SterilEnz-11/G, 2G–616–TC–5X5, to effectively serve as a complete microbial barrier, therefore maintaining its sterile environment until it reaches its point of end use. SterilEnz–11/G, 2G–616–TC–5X5 was challenged with the test microorganism *Bacillus subtilis* (ATCC # 6633).

2.0 REFERENCES

The study was conducted based upon the following references:

2.1 United States Pharmacopeia 32, National Formulary 27, 2009. <1207> Sterile Product Packaging – Integrity Evaluation.

2.2 ISO 11607 Packaging for Terminally Sterilized Medical Devices, 2006.

2.3 United States Pharmacopeia 32, National Formulary 27, 2009. <71> Sterility Tests.

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

3.0 COMPLIANCE

Although this study is non–GLP, it was conducted according to the accredited Quality System in effect at Toxikon, including 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 and GMPs.

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:

Test Article Name: SterilEnz–11/G, 2G–616–TC–5X5
CAS/Code #: Not Supplied by Sponsor (N/S)
Lot/Batch #: A0109–017
Physical State: N/S
Color: N/S
Expiration Date: N/S
Density: N/S
Stability: N/S

Solubility: N/S
pH: N/S
Storage Conditions: Room Temperature
Safety Precautions: Standard Toxikon Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

To evaluate the test article's ability to maintain its sterile environment, the complete outer surface of the sealed pouch containing the connector was challenged by immersing it in a buffer solution containing a known concentration of the indicator organism *Bacillus subtilis* (ATCC # 6633). The test articles were immersed for approximately 1 minute and the challenge suspension was allowed to remain on the test article for at least 4 hours. The connector (located inside the sealed pouch) and the entire internal surface area of the pouch were then tested for sterility. The test organism was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

6.0 JUSTIFICATION OF TEST SYTEMS AND ROUTE OF ADMINISTRATION

This test system is most commonly utilized within the industry for microbial ingress/package challenge testing. The test system provides a qualitative method for the evaluation of sterility maintenance post microorganism challenge.

7.0 EXPERIMENTAL DESIGN AND DOSAGE

7.1 Challenge Organism Preparation:
The bacterial culture was grown overnight in TSB at 37 ± 1 °C. Post incubation, the culture was pelleted by centrifugation, the supernatant was removed and the pellet resuspended in Phosphate Buffered Solution (PBS). This wash step was repeated twice. A spectrophotometer (0.26–0.32 OD_{475}) was to measured the concentration and each bacterial culture was adjusted to a concentration of approximately 10^8 Colony Forming Units (CFU)/mL.

7.2 The concentration of the challenge inoculum was verified by performing plate counts using the spread plate method. Decimal dilutions of the culture were prepared (utilizing sterile PBS as the diluent). The plates were incubated at 30–35 °C for up to 7 days. This determined the actual concentration of the test organism exposed to the test article package.

7.3 Test Article Preparation:
Twenty five (25) test articles were aseptically transferred to a Class 100 laminar flow hood. Twenty four of these test articles were inoculated as received from the Sponsor, and one was not inoculated (served as a negative control). Four (4) additional test articles were prepared by piercing them several times with a sterile needle, compromising the integrity of the package. These four test articles served as positive controls.

7.4 Test Article Challenge:
Twenty four (24) test articles (TA) and four (4) positive control test articles (PCTA) were challenged by immersing each test article in the buffer solution containing at least 1×10^5 Colony Forming Units (CFU)/mL of the challenge microorganism.

7.5 The TAs and the PCTAs were allowed to remain in the bath for up to 1 minute. The test articles were laid out to dry under a laminar flow hood. The test articles were challenged for 4-8 hours.

7.6 Disinfecting Procedure:
After the contact period at room temperature, the area of the outer pouch that is most suitable for opening the package was wiped down with a disinfectant. This was repeated three (3) times prior to opening the package.

7.7 Recovery and Incubation Procedures:

7.7.1 The entire internal surface area of the pouch and the connector were tested for sterility. Trypticase Soy Broth (TSB) was used to fill the pouch and remained in the pouch for 30 minutes. The media was transferred to a sterile flask and incubated at 30–35 °C for 14 days.

7.7.2 The vessels were examined for growth daily for 14 days (except weekends and holidays). The contents of the vessels were examined for macroscopic evidence of microbial growth, such as the development of turbidity.

7.7.3 The recovery procedure applied to non–bacteriostatic test articles. The PCTA in this study will determine if the test article was non–bacteriostatic.

7.8 Test System Controls:

7.8.1 An uninoculated test article served as the negative control. The contents of the test article (1 unit) was tested as in Section 7.0 in order to verify sterility of the test article.

7.8.2 Sterility and growth promotion properties of TSB media were verified prior to initiation of the assay.

8.0 EVALUATION CRITERIA

8.1 The test media should exhibit satisfactory growth promotion characteristics.

8.2 The test article should be sterile prior to initiation of the assay.

8.3 Growth should be observed for the positive control articles.

8.4 The concentration of the challenge organism should be confirmed to be at least 1×10^5 CFU/mL.

8.5 No growth should be observed for any of the test articles.

8.6 In case of any questionable turbidity in the test article the media is eluted into a clear plastic tube to further observe turbidity in reference to the positive control and a Gram's stain is performed to check the presence of the challenge test organism used in this study. The test article fails to meet the requirements of the assay if growth is observed and identified to be the test organism.

8.7 The study and its design employed methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

9.0 RESULTS

The results are summarized in Table 1.

10.0 CONCLUSION

A Microbial Barrier Performance Test was carried out to evaluate the ability of the test article, SterilEnz-11/G, 2G–616–TC–5X5, to effectively serve as a complete microbial barrier, therefore maintaining its sterile environment until it reaches its point of end use. SterilEnz–11/G, 2G–616–TC–5X5 was challenged with the test microorganism *Bacillus subtilis* (ATCC # 6633). The complete outer surface of the sealed pouch containing the connector was challenged by immersing it in a buffer solution containing 1 x 10^5 CFU/mL of the indicator organism *Bacillus subtilis*. Twenty four (24) test articles (TA) and four (4) positive control test articles (PCTA) were immersed for approximately 1 minute and the challenge suspension was allowed to remain on the test article for 4 hours. After the contact period at room temperature, the area of the outer pouch that is most suitable for opening the package was wiped down with a disinfectant. This was repeated three (3) times prior to opening the package. The entire internal surface area of the pouch and the connector were tested for sterility. Trypticase Soy Broth (TSB) was used to fill the pouch and remained in the pouch for 30 minutes. The media was transferred to a sterile flask and incubated at 30–35 °C for 14 days.

The contents of the vessels were examined for macroscopic evidence of microbial growth daily for 14 days. Over this 14 day period, there was no evidence of microbial growth. Therefore, it is concluded that the SterilEnz-11/G, 2G–616–TC–5X5 serves as a complete microbial barrier and its sterile environment will remain sterile following a worst-case microbial challenge.

11.0 RECORDS

11.1 Original raw data are archived at Toxikon Corporation.

11.2 A copy of the final report and any report amendments is archived at Toxikon Corporation.

11.3 The original final report is forwarded to the Sponsor.

11.4 All test articles were disposed of by Toxikon, per Sponsor's request.

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.

TABLE 1
Results

Test Article: SterilEnz–11/G, 2G–616–TC–5X5

Lot/Batch #: A0109–017

Test Article																								
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
3	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W
4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

– = Negative, No growth W = Weekend

Negative Control Test Article	
Day	25
3	W
4	–
5	–
7	–
14	–

– = Negative
W = Weekend

Positive Control Test Article				
Day	26	27	28	29
3	W	W	W	W
4	+	+	+	+
5	+	+	+	+
7	+	+	+	+
14	+	+	+	+

+ = Positive, turbid media, growth of visible bacteria
W = Weekend

APPENDIX I
Software Systems

Software	Use
Adobe Acrobat 8 Professional	Document preparation
DocuKnowledge 3.0	Lotus Domino–based document management system used for SOPs
Lotus Domino Rel. 5	Client–server application for sponsor, sample, test codes, and quotation management application databases
MS Office 2007 Small Business Suite	Business software (suite includes Word, Excel, PowerPoint, Outlook, Publisher, Office tools)
Rees Centron SQL System 2.0	Environmental Monitoring and Metrology System
UV_WINLAB V.2.85.04	Spectrophotometer for absorption measurement