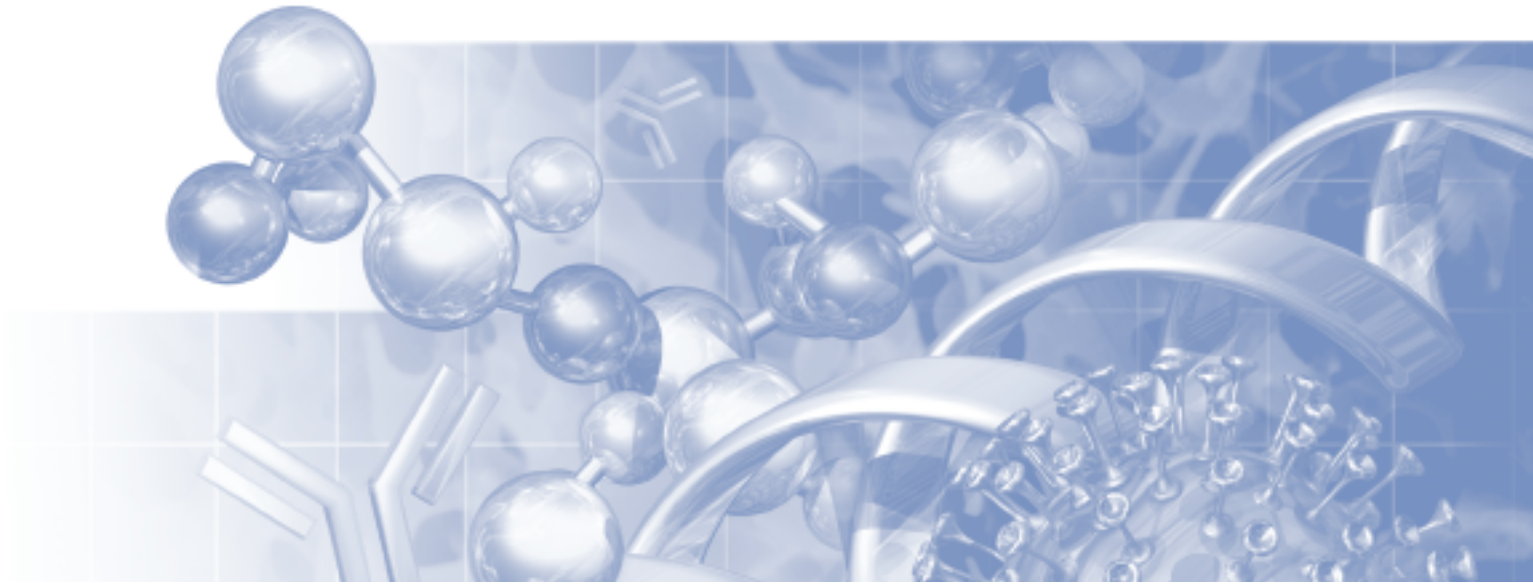
PALL Life Sciences

Validation Guide

USTR 2267

Mustang® E Membrane Chromatography Capsules



*Filtration. Separation. Solution.*SM

Contents

1. Overview

1.1 Introduction

Mustang E membranes, capsules and cartridges are designed to remove residual endotoxin (lipopolysaccharides) from water, water for injection (WFI) and from buffers used in the purification of biomolecules in downstream bioprocessing applications.

Mustang E membranes are manufactured with a proprietary Pall polyethersulfone modified with a highly cross linked quaternized amine charge polymer coating based on polyethyleneimine.

Mustang E units contain 3 layers of pleated membrane and are available as fully disposable capsules housed within either a polyetherimide or polypropylene shell. Some of the validation work presented here was done using a Mustang E CL05 capsule (10 mL membrane volume). The product housing has since changed and the product is now known as Mustang E CLM05. As only the housing changed and not the membrane element, the validation data herein is applicable to the new capsule. Further details about Mustang E capsules can be found in the product data sheet, reference Pall publication USD 2280.

The purpose of this report is to summarize the tests that were performed to qualify the performance of Mustang E membrane and units under standard conditions. This testing program included:

- Determination of Endotoxin Dynamic Binding Capacities
- Determination of Flow Characteristics at different applied pressures
- Endurance to Autoclave Sterilization
- Endurance to Sanitization using sodium hydroxide
- Extractables Testing
- Biological Reactivity Tests on Mustang E membrane

1.2 Summary of Conclusions

1.2.1 Determination of Endotoxin Dynamic Binding Capacities

The tests performed demonstrate that Mustang E capsules exhibit high endotoxin binding capacities, and are therefore suitable for downstream bioprocessing applications for the capture and removal of endotoxin from water, WFI and buffer feedstreams.

The presence of proteins, nucleic acids or any other biological compound may influence the performance, and it is therefore recommended that the user evaluate Mustang E capsules using specific process fluids under standard operating conditions.

1.2.2 Determination of Flow Characteristics

The flow rate of Tris-HCl buffer at an applied pressure of 1.0 bar (14.5 psi) was measured using typical Mustang E capsules. The results can be used to assist the user in sizing systems that employ Mustang E capsules when used with process fluids of similar viscosities.

1.2.3 Endurance to Autoclave Sterilization

Endotoxin dynamic binding capacity tests have been performed to demonstrate that autoclave sterilization for 30 minutes at 121 °C (250 °F) does not influence the performance of Mustang E capsules.

Warning: Mustang E products should not be used with fluids that are incompatible with the materials of construction. Incompatible fluids are those that chemically attack, soften, stress crack or adversely affect the materials of construction in any way. Fluids that should not be used include cleaning agents and fluids containing organic solvents such as alcohol.

1.2.4 Compatibility with Sodium Hydroxide

Tests performed with Mustang E capsules in 1M NaOH solution for 60 minutes at 20 °C (68 °C) demonstrated that no micro-cracking was observed in the cartridge hardware and the endotoxin dynamic binding capacity of the membrane was also found to be unaffected.

1.2.5 Extractables Testing

The amount of non-volatile residue extracted from preconditioned Mustang E cartridges was found to be extremely low. Actual service will impose different conditions, different exposure times, temperature, liquid purity, etc. Evaluation under process conditions is therefore also recommended.

1.2.6 Biological Reactivity Tests on the Mustang E Membrane

Mustang E capsules met the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI Plastics (50 °C [122 °F]).

2. Product Specifications

2.1 Introduction

Four CL3MSTGEP1, two CLM05MSTGEP1* and one NP6MSTGEP1 capsules were tested for endotoxin dynamic binding capacity in saline. Three capsules (part number CL3MSTGP1) were autoclaved at the following conditions: (i) 125 °C (257 °F) for 60 minutes, (ii) 125 °C (257 °F) for 30 minutes and 121 °C (249 °F) for 30 minutes. One capsule (part number CLM05MSTGQP1) was autoclaved at 121 °C (249 °F) for 30 minutes. One capsule (part number CL3MSTGEP1) was exposed to 1M NaOH for one hour before testing for its ability to remove endotoxin from saline.

The objective of this series of experiments was to determine the breakthrough endotoxin removal levels for capsule/cartridge formats containing three layers of Mustang E membrane. The effect of both autoclaving and subjection to 1M NaOH on the performance of Mustang E capsules was also determined.

When the original testing was conducted, the part number at that time was CL05MSTGEP1. It has since been changed to CLM05MSTGEP1. The two part numbers represent equivalent products with the same membrane element, the same materials of construction, and the same overall performance..

2.2 Summary of Methods

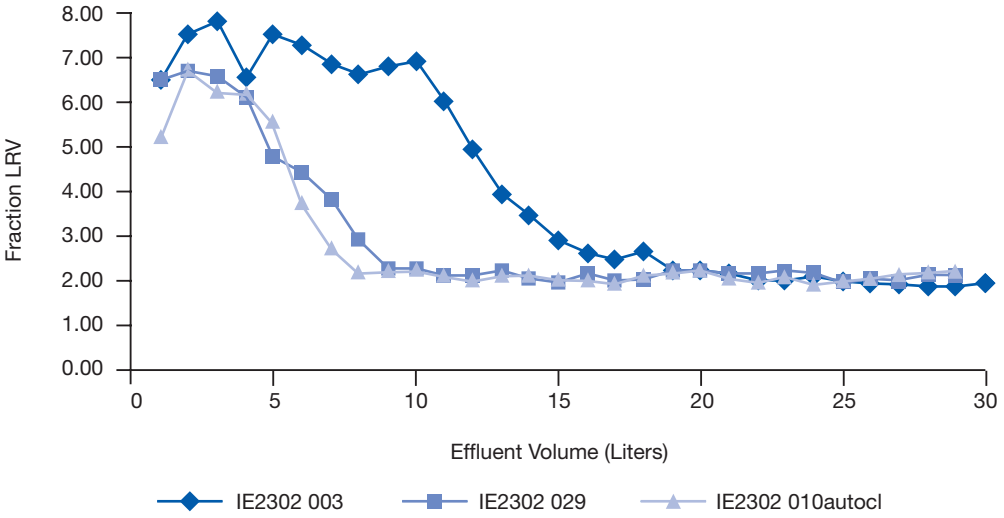
Typical Mustang E capsules from standard production lots were used for the tests (part numbers CLM05MSTGEP1, CL3MSTGEP1 and NP6MSTGEP1). Determinations of the binding capacity for endotoxin was performed as described below. All test solutions and reference standards were made up according to the suppliers' specifications.

Test capsules were equilibrated with 0.9% saline solution. Flow rates were adjusted to 500 mL/min for CL3MSTGEP1 and CLM05MSTGEP1 capsules and 1.0 L/min for the NP6MSTGEP1 capsule. A 50 mL fraction was collected into 50 mL pyrogen free polystyrene tube as a control. Further 50 mL fractions were collected every 2 minutes for the CLM05MSTGEP1 and CL3MSTGEP1 capsule and every 1 minute for the NP6MSTGEP1 capsule. A total of 25 fractions were collected for the CL3MSTGEP1 and CLM05MSTGEP1 capsules and 70 fractions for the NP6MSTGEP1 capsule.

2.3 Results

Figure 1

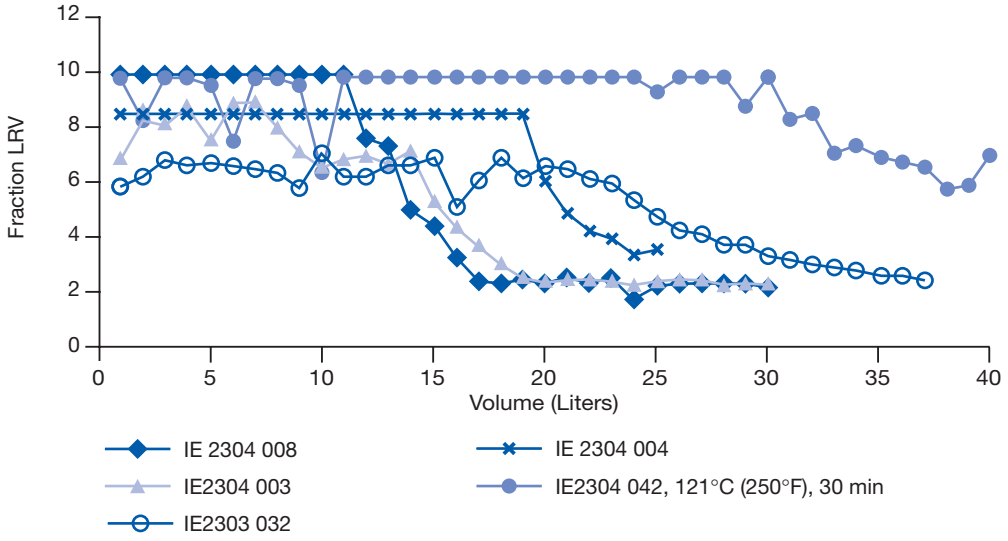
Mustang E Capsules CLM05 Format (Fraction LRV)



Log Reduction Value (LRV) for endotoxin removed by CLM05MSTGEP1 capsules. Amount of actual endotoxin challenge for each capsule tested: IE2302003; 2.85E+04 EU/mL, total challenge 8.55E+08 EU; total challenge 1 IE2302029; 3.54E+04 EU/mL, total challenge 1.06E+09; IE2302010 [autoclaved at 121 °C (249 °F) for 30 minutes]; 3.54E+04 EU/mL, total challenge 1.06E+009 EU.

Figure 2

Fraction LRV Mustang E Capsule CL3 Challenged with Endotoxin in 0.9% Saline



Fraction Log Reduction Value (LRV) for endotoxin removed by CL3MSTGEP1 capsules. Amount of actual endotoxin challenge for each capsule tested: IE2304008; 8.17E+04 EU/mL, total challenge 3.27E+09 EU; IE2304003; 5.60E+04 EU/mL, total challenge 2.24E+09 EU; IE2304004; 3.08E+04 EU/mL, IE2303032; 3.79E+04 EU/mL, total challenge 1.52E+09 EU; IE2304042 [autoclaved at 121 °C (249 °F) for 30 minutes]; 5.90E+04 EU/mL, total challenge 2.36E+09 EU.

Table 1

Dynamic Endotoxin Binding Capacity for CLM05MSTGEP1 Capsule (10 mL Total Membrane Volume)

Lot No.	EU/CLM05	EU/mL
IE2302003	3.70E+08	3.70E+07
IE2302029	2.40E+08	2.40E+07
AVE	3.05E+08	3.05E+07

Table 2

Dynamic Endotoxin Binding Capacity for CL3MSTGEP1 Capsule (44 mL Total Membrane Volume)

Lot No.	EU/CL3	EU/mL
IE2304008	1.22E+09	2.77E+07
IE2304003	8.10E+08	1.84E+07
IE2304004	6.16E+08	1.40E+07
IE2303032	2.88E+09	6.55E+07
AVE	1.38E+09	3.14E+07

Table 3

Dynamic Endotoxin Binding Capacity for NP6MSTGEP1 Capsule (158 mL Total Membrane Volume)

Lot No.	NP6	EU/mL
PB788	1.72E+09	1.09E+07

Table 4

Average Endotoxin Dynamic Binding Capacity Based on Membrane Volume from Tables 1, 2 and 3

Mustang E Capsules	Eu/mL
CLM05-IE2302003	3.70E+07
CLM05-IE2302023	2.40E+07
CL3-E2304008	2.77E+07
CL3-IE2304003	1.84E+07
CL3-IE2304004	1.40E+07
CL3-IE2303032	3.17E+07
NP6-PB788	1.09E+07
AVERAGE	2.34E+07
STD. DEVIATION	9.51E+06

2.4 Conclusions

The calculated membrane volumes for three layers of Mustang E membrane in the three capsules tested are: CLM05 = 10 mL, CL3 = 44 mL, and NP6 = 158 mL. When data from the three capsules was tested using the calculated membrane values, there was very good agreement for the CLM05 and CL3 data (Table1; 3.05E+07 EU/mL versus Table 2; 3.14E+07). The only 10-inch capsule tested (Table 3; 1.09E+07EU/mL) gave a value about one-third of the other two capsules. All seven of the normalized values from two CLM05, four CL3 and one NP6 capsules were then averaged and the results given in Table 4. The data described in Table 1, 2 and 3 are supporting the claim of a minimum endotoxin retention of 4E+06 EU/mL of media.

3. Determination of Flow Characteristics

3.1 Introduction

The aim of this series of tests was to determine the flow characteristics of typical Mustang E capsules operated at 1.0 bar (14.7 psi) upstream pressure using an aqueous test fluid.

3.2 Summary of Methods

Typical Mustang E capsules from production were used for the tests (part numbers CLM05MSTGEP1 and CL3MSTGEP1). The test fluid used was 10 mM MES buffer at pH 5.5, temperature 20 °C (68 °F). The fluid was pumped through the test capsules at set upstream pressure.

Throughout each test the capsule outlets were maintained at atmospheric pressure. The flow rate on the downstream side of the capsule was measured over a one-minute interval.

3.3 Results

Results of Tris-HCl buffer versus applied upstream pressure are shown in Table 5.

Table 5

Flow Rates at 1.0 bar (14.7 psi) Upstream Pressure for Mustang E Capsules

Part Number	Test Pressure Bar (psi)	Flow Rate mL/min (L/hr)
CLM05MSTGEP1	1.0 (14.7)	2,500 (150)
CL3MSTGEP1	1.0 (14.7)	8,500 (510)

3.4 Conclusions

The flow characteristics quoted in this report can be used to assist in sizing systems employing Mustang E capsules when used with process fluids of similar viscosities.

4. Endurance to Autoclave Sterilization

4.1 Introduction

The purpose of these tests was to demonstrate that either a 30 or 60 minute autoclave cycle at 121 °C (250 °F) or at 125 °C (257 °F) would not influence the performance of Mustang E capsules, as determined using a standard endotoxin challenge solution before and after autoclaving.

Warning: Mustang E products should not be used with fluids that are incompatible with the materials of construction. Incompatible fluids are those that chemically attack, soften, stress crack or adversely affect the materials of construction in any way. Fluids that should not be used include cleaning agents and fluids containing organic solvents such as alcohol.

4.2 Summary of Methods

Typical Mustang E capsules from production were used for these tests (part numbers CLM05MSTEQP1 and CL3MSTGEP1). Samples were removed from their packaging, the inlet and outlet connections loosely wrapped, and then the capsules were autoclaved at 121 °C (250 °F) or 125 °C (257 °F) for either 30 or 60 minutes.

Following autoclave, the cooled capsules were tested for endotoxin binding capacity according to the procedure described previously in Section 2.2. Non-autoclaved samples were also tested as a control.

4.3 Results

The results are shown in Table 6. It was found that the binding capacity of endotoxin bound on autoclaved and non-autoclaved samples was very similar, indicating that 30 or 60 minute autoclave cycles at either 121 °C (250 °F) or 125 °C (257 °F) has very little effect on the endotoxin binding capacities of Mustang E capsules.

Table 6

Effect of Autoclaving on Mustang E Capsule Endotoxin Dynamic Binding Capacity

Capsule Type	Lot No.	Autoclave Conditions	EU/capsule	EU/mL
CLM05MSTGEP1	IE2302010	121 °C (250 °F) for 30 min	1.02E+09	1.02E+08
CL3MSTGEP1	IE2304042	121 °C (250 °F) for 30 min	2.89E+09	7.22E+07
CL3MSTGEP1	IE2304057	125 °C (257 °F) for 60 min	1.20E+08	2.73E+06
CL3MSTGEP1	IE2304013	125 °C (257 °F) for 30 min	3.26E+08	7.41E+06

4.4 Conclusions

Mustang E capsules can be autoclaved for 30 minutes at 121 °C (250 °F) without the performance of the capsule being influenced, as demonstrated using a spiked endotoxin assay test.

5. Compatibility with Sodium Hydroxide

5.1 Introduction

Users of Mustang E capsules may wish to use sodium hydroxide for sanitization. The aim of these tests was to determine if Mustang E capsules are compatible with 1M NaOH for 60 minutes at 20 °C (68 °F). There were two aspects to this testing as follows:

- Test to determine if 1M NaOH caused any visible damage to the cartridge hardware.
- Test to determine if exposure to 1M NaOH influenced the dynamic endotoxin binding capacity of the Mustang E membrane.

5.2 Summary of Methods

Examination of Cartridge Hardware Following Exposure to 1M NaOH

Tests previously undertaken on the component cartridge elements from Mustang capsules show that limited exposure (30 minutes) to 1M NaOH produces no chemical induced stress cracking within the cartridge hardware. Reference USTR 2101 Validation Guide for Mustang Q capsules.

Determination of Endotoxin Binding Capacity before and after Exposure to 1M NaOH

Mustang E capsules were used for these tests (part number CL3MSTGEP1). The endotoxin dynamic binding capacity was determined on samples before and after exposure to 1M NaOH solution for 60 minutes at 20 °C (68 °F). The method used for determining endotoxin binding capacity is that previously described in Section 2.2.

5.3 Results

Table 7

Effect of Exposure to 1M NaOH for 1 Hour on CL3MSTGEP1 Capsule's Dynamic Endotoxin Binding Capacity (Endotoxin Challenge at 2.95E + 04 EU/mL)

Lot No.	EU/capsule	EU/mL	EU/mL (Average for batch # IE2304)
IE2304015	6.49E+08	1.62E+07	2.003E+07

The results of the dynamic endotoxin binding capacity tests shown in Table 7 demonstrate that exposure to 1M NaOH for 60 minutes at 20 °C (68 °F) had no significant effect on membrane performance.

5.4 Conclusions

Tests performed with Mustang E capsules in 1M NaOH solution for 60 minutes at 20 °C (68 °F) demonstrated that the endotoxin dynamic binding capacity of the membrane was also found to be unaffected.

6. Extractables Testing

6.1 Introduction

The purpose of this series of tests was to quantify and analyze the amount of material that can be extracted from Mustang E units by water at ambient temperature, 20 ± 5 °C (68 ± 41 °F).

6.2 Summary of Methods

Extraction in water was undertaken on 3 samples of each of the following Mustang E units, part numbers CLM05MSTGEP1, CL3MSTGEP1 and AB1MSTGE7PH4. Prior to the extraction the capsules were preconditioned by flushing, at a flow rate of 1500 mL/min, with:

- 1M NaOH (minimum 3000 mL)
- 1M NaCl (minimum 3000 mL)
- 18 MΩ water (until the downstream pH and conductivity measurements were the same as the upstream measurements)

Following preconditioning, the extraction procedure was performed by recirculating 1500 mL of 18 MΩ water through the cartridge for two hours at a flow rate of 400 mL/min for the capsules part number CLM05MSTGEP1 and CL3MSTGEP1. On the 10" cartridge (part number AB1MSTGE7PH4), the extraction was performed by reciprocation for 4 hours at 20 °C (68 °F). After the extraction period, the sample was concentrated to approximately 100 mL using a rotoevaporator. A volume of the sample was then evaporated to dryness and the non-volatile residue (NVR) was determined gravimetrically. The results were corrected to express the NVR for the entire extraction volume used. A sample of the NVR was analyzed by Fourier Transform Infra Red Spectroscopy (FTIR).

6.3 Results

The amount of extractables obtained from the Mustang E capsules tested is shown in Table 8.

Table 8

Non-volatile Aqueous Extractables Obtained using Mustang E Capsules, Part Numbers CLM05MSTGEP1, CL3MSTGEP1 and NP6MSTGEP1

Capsule Part Number	Capsule or Cartridge Sample Number	Non-Volatile Residue
CLM05MSTGEP1	1	2 mg
	2	2 mg
	3	1 mg
Average Value		**2 mg**
CL3MSTGEP1	1	5 mg
	2	4 mg
	3	5 mg
Average Value		**5 mg**
NP6MSTGEP1	1	101 mg
	2	73 mg
	3	118 mg
Average Value		**97 mg**

6.4 Conclusions

The levels of aqueous extractables determined for preconditioned Mustang E devices were found to be low. The levels measured for typical capsules and cartridges, part numbers CLM05MSTGEP1, CL3MSTGEP1 and AB1MSTGE7PH4 ranged respectively from 2 mg to 97 mg. The infrared spectroscopy showed that the materials extracted were composed primarily of oligomers and additives/processing aids related to the polymers used in the cartridge.

Actual service will impose different conditions, such as different exposure times, temperature, liquid purity, etc. Evaluation under process conditions is therefore also recommended.

7. Biological Reactivity Tests on the Mustang E Membrane

7.1 Introduction

The purpose of this study was to evaluate the biological suitability of the membrane used in Mustang E units.

7.2 Summary of Methods

The membrane used to manufacture Mustang E capsules was used for the tests. The membrane was pretreated by exposure to different sterilization procedures:

- Autoclaving at 121 °C (250 °F) for 60 minutes
- Exposure to gamma irradiation

The tests were performed in accordance with the Biological Reactivity Tests *in vivo* for Class VI Plastics (50 °C [122 °F]) as described in the current *United States Pharmacopeia.* The tests were conducted by Toxikon Corporation, Bedford, USA.

The testing procedures described in the USP include:

- Injection of extracts of the test article
- Implantation of the test article into animal tissue

The four extracting media listed in the USP simulate parenteral solutions and body fluids.

These include:

- Sodium Chloride for Injection
- 1 in 20 Solution of Alcohol in Sodium Chloride Injection
- Polyethylene Glycol 400
- Vegetable Oil (sesame or cottonseed oil)

The USP states that extracts may be prepared at one of three standard conditions: 50 °C (122 °F) for 72 hours, 70 °C (158 °F) for 24 hours, or 121 °C (250 °F) for 1 hour. Mustang E membrane was tested at 50 °C (122 °F) for 72 hours.

Acute Systemic Injection Tests (ACIT)

An Acute Systemic Injection Test was performed to evaluate the potential of a single injection of an extract to produce systemic toxicity. Sodium Chloride Injection and a 1 in 20 Solution of Alcohol in Sodium Chloride Injection were injected intravenously. Vegetable oil extract and Polyethylene Glycol 400 extract were injected intraperitoneally.

Intracutaneous Tests

An Intracutaneous Test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. All four of the extracts listed above were used for these tests.

Implantation Tests

Implantation tests were also performed to subject the Mustang E membrane to the most stringent conditions included in the USP.

Physiochemical Test for Plastics

The membrane was extracted in sterile Water for Injection (WFI) and the extract was tested for non-volatile residue, heavy metals and buffering capacity. Results are correlated against applied USP23 standards.

7.3 Results

No biological response was observed in any of the tests performed and therefore the preconditioned Mustang E membrane passed all of the tests specified.

7.4 Capsule Components

Capsule components were tested and meet the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI Plastics (50 °C [122 °F]). (Refer to Pall's Kleenpak™ Capsules Validation Guide [USTR 2099] and Novasip™ Capsules Validation Guide [USTR 1666].)

7.5 Conclusions

Mustang E capsules meet the requirements of the USP Biological Reactivity Tests *(in vivo)* for Class VI Plastics (50 °C [122 °F]).

PALL Life Sciences

United States
800.717.7255 toll free (USA)
516.484.5400 phone
516.801.9548 fax
biopharm@pall.com E-mail

Europe
+41 (0)26 350 53 00 phone
+41 (0)26 350 53 53 fax
LifeSciences.EU@pall.com E-mail

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Visit us on the Web at www.pall.com/biopharm
E-mail us at biopharm@pall.com

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