Validation Guide

Pall Omega™ Membrane Cassettes

Centramate™, Centrasette™, Maximate™, Maxisette™ Membrane Cassettes
Table of Contents

1 Purpose of this Document ................................................................. 1
  1.1 Validating Filtration Processes — General Concepts ......................... 1
  1.2 Installation Qualification (IQ) .......................................................... 1
  1.3 Operational Qualification (OQ) ......................................................... 1
  1.4 Performance Qualification (PQ) ....................................................... 2

2 Product Specifications ....................................................................... 3
  2.1 Packaging .......................................................................................... 3
  2.2 Part Numbers ..................................................................................... 3
  2.3 Serial Numbers .................................................................................. 5
  2.4 Materials of Construction ................................................................. 5
    2.4.1 Membrane ...................................................................................... 5
    2.4.2 Screens .......................................................................................... 5
    2.4.3 Spacers .......................................................................................... 5
    2.4.4 Encapsulant .................................................................................... 5
    2.4.5 Gaskets .......................................................................................... 5
  2.5 Dimensions ....................................................................................... 5
  2.6 Operating Pressures and Temperatures ............................................. 6
  2.7 Water Flux Ranges ............................................................................ 6
    2.7.1 Water Flux Specifications for Omega Membranes ....................... 7
    2.7.2 Typical Water Flux Values for Omega Membrane Cassettes .......... 7
  2.8 Membrane Solute Passage Specifications ......................................... 8
  2.9 Test Solute Concentrations and Detection Methods ......................... 9
  2.10 Shelf Life of New and Used Cassettes ............................................. 9
  2.11 Membrane Integrity—Forward Flow Test Values ............................ 10
  2.12 Chemical Compatibility—Sodium Hydroxide, 0.05 – 1 N, 20 °C .......... 10
  2.13 Chemical Compatibility — General ............................................... 11
  2.14 Protein Binding Characteristics ...................................................... 12

3 Validation Procedures ....................................................................... 14
  3.1 Solvent Compatibility ....................................................................... 14
    3.1.1 Introduction .................................................................................. 14
    3.1.2 Scope ........................................................................................... 14
    3.1.3 Summary of Method................................................................. 14
  3.2 Retention Characteristics — Dextran Selectivity Procedure ............... 16
    3.2.1 Summary of Method................................................................. 16
  3.3 Extractables Test ............................................................................. 19
    3.3.1 Introduction ................................................................................ 19
    3.3.2 Summary of Method................................................................. 19
    3.3.3 Results ....................................................................................... 19
    3.3.4 Conclusions .............................................................................. 20
6.9 Integrity Testing ......................................................................................................... 35
  6.9.1 System Integrity Test .......................................................................................... 36
  6.9.2 Membrane Integrity Test .................................................................................. 36
6.10 Method for Determining Water Flux and Normalized Water Permeability ............. 37
6.11 Cleaning the System and Cassettes ........................................................................ 37
  6.11.1 Cross Flow (Retentate Flow Rate) ..................................................................... 37
  6.11.2 Cleaning Agent and Concentration .................................................................... 37
  6.11.3 Temperature ...................................................................................................... 37
  6.11.4 Time .................................................................................................................. 37
6.12 Recommended Cleaning Procedures ......................................................................... 38
6.13 Cleaning Study Protocol ........................................................................................ 38
7 System Characteristics ............................................................................................. 39
  7.1 System Hardware and Design Considerations ......................................................... 39
    7.1.1 Holder .............................................................................................................. 39
    7.1.2 Pressure Gauges (Transducers) ......................................................................... 39
    7.1.3 Valves ................................................................................................................. 40
    7.1.4 Fittings Package for Cassette Holders ................................................................. 40
    7.1.5 Feed Reservoir .................................................................................................. 40
    7.1.6 Feed Pump ......................................................................................................... 41
    7.1.7 Piping .................................................................................................................. 41
    7.1.8 Heat Exchanger ................................................................................................. 41
  7.2 Cassette and System Hold-Up Volume ...................................................................... 41
    7.2.1 Cassette Hold-up Volume .................................................................................. 42
    7.2.2 System Hold-up Volume ................................................................................... 42
8 Method Details ................................................................................................---------- 43
  8.1 Procedure for Determination of Extractables from Omega Membrane Cassettes ......... 43
    8.1.1 Equipment .......................................................................................................... 43
    8.1.2 Reagents and Materials .................................................................................... 43
    8.1.3 Preparation of Apparatus and Materials ............................................................. 43
    8.1.4 Extraction Procedure ....................................................................................... 43
  8.2 Procedure for Determination of Nonvolatile Residue ................................................. 44
  8.3 Effectiveness of the Flushing Protocol for Removing Storage Agents ......................... 44
  8.4 Determining Solute Passage on Membrane Disk ....................................................... 45
    8.4.1 Introduction ........................................................................................................ 45
    8.4.2 Materials and Equipment ................................................................................... 46
    8.4.3 Chemicals .......................................................................................................... 46
    8.4.4 Preparation of Test Solutions ......................................................................... 46
    8.4.5 Procedure ......................................................................................................... 46
    8.4.6 Analysis and Interpretation of Data ............................................................... 47
  8.5 Determining Solute Passage on Membrane Cassettes ............................................... 47
    8.5.1 Introduction ....................................................................................................... 47
9 Detection Methods ........................................................................................................49
  9.1 Detection Methods for Cleaning Agents .................................................................49
    9.1.1 Sodium Hydroxide ..........................................................................................49
  9.2 Detection Methods for Storage Agents .................................................................49
    9.2.1 Materials and Equipment .............................................................................49
    9.2.2 Sodium Azide ................................................................................................49
    9.2.3 Sample Preparation .......................................................................................49
    9.2.4 Sodium Azide Detection ...............................................................................49
    9.2.5 Glycerin ........................................................................................................50
  9.3 Endotoxin Assay Procedure — ThermoMax Chromogenic Assay .........................50
    9.3.1 Introduction ....................................................................................................50
    9.3.2 Detection Limit ...............................................................................................50
    9.3.3 Materials ........................................................................................................50
    9.3.4 Reagents ........................................................................................................51
    9.3.5 Endochrome* Reagent ..................................................................................51
    9.3.6 ThermoMax Setup .........................................................................................51
    9.3.7 LAL Assay ......................................................................................................52
    9.3.8 Interpreting Results .......................................................................................52
    9.3.9 Analyst Qualification .....................................................................................52

10 Biosafety Test Results ..............................................................................................53
  10.1 Test Results — Biological Test for Plastics ..........................................................53
  10.2 Test Results — Cytotoxicity Test ........................................................................58
  10.3 Test Results — Hemolysis ..................................................................................61
  10.4 Test Results — Endotoxin Levels and Total Oxidizable Substances .....................64

* Endochrome is a trademark of Charles River Laboratories.
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1:</td>
<td>Example of Information Printed on the Side of Cassettes</td>
<td>3</td>
</tr>
<tr>
<td>Figure 2:</td>
<td>Part Number Code</td>
<td>3</td>
</tr>
<tr>
<td>Figure 3:</td>
<td>Cassette Suspended Screen Channel Configurations</td>
<td>6</td>
</tr>
<tr>
<td>Figure 4:</td>
<td>Typical Normalized Water Permeability for Membrane Cassettes compared to a Disc Membrane</td>
<td>7</td>
</tr>
<tr>
<td>Figure 5:</td>
<td>Nonspecific Adsorption Characteristics of Three Membrane Materials</td>
<td>13</td>
</tr>
<tr>
<td>Figure 6:</td>
<td>Example of Dextran Selectivity Curves for Omega Membranes</td>
<td>16</td>
</tr>
<tr>
<td>Figure 7:</td>
<td>Molecular Weight of Dextran Versus Retention Time on TSK G4000PW Column</td>
<td>17</td>
</tr>
<tr>
<td>Figure 8:</td>
<td>Example of Refractive Index Detector Response Versus Retention Time of Dextran from TSKG4000PW Column</td>
<td>18</td>
</tr>
<tr>
<td>Figure 9:</td>
<td>Example of Membrane Percent Rejection versus Dextran Molecular Weight Curve</td>
<td>18</td>
</tr>
<tr>
<td>Figure 10:</td>
<td>Infrared Spectrum of Nonvolatile Residue (Water Extract) as Smear on KBr</td>
<td>20</td>
</tr>
<tr>
<td>Figure 11:</td>
<td>Infrared Spectrum of Nonvolatile Residue (25% Ethanol Extract) as Smear on KBr</td>
<td>20</td>
</tr>
<tr>
<td>Figure 12:</td>
<td>Concentration of Glycerin and Sodium Azide in the Flush Water from a New Cassette</td>
<td>22</td>
</tr>
<tr>
<td>Figure 13:</td>
<td>Results of Low Volume, Multiple Recirculation Flushing to Remove Sodium Azide and Glycerin</td>
<td>23</td>
</tr>
<tr>
<td>Figure 14:</td>
<td>Example of a Certificate of Test</td>
<td>27</td>
</tr>
<tr>
<td>Figure 15:</td>
<td>Example of Box Label</td>
<td>28</td>
</tr>
<tr>
<td>Figure 16:</td>
<td>Example of the Information Printed on Omega Cassettes</td>
<td>28</td>
</tr>
<tr>
<td>Figure 17:</td>
<td>Pall Manual-Torque Cassette Holders and Torquing Sequence</td>
<td>33</td>
</tr>
<tr>
<td>Figure 18:</td>
<td>TFF System Schematic with Feed/Retentate Flow Path</td>
<td>35</td>
</tr>
<tr>
<td>Figure 19:</td>
<td>Membrane Integrity Test Setup Using a Pall Integrity Analyzer</td>
<td>36</td>
</tr>
<tr>
<td>Figure 20:</td>
<td>Typical Tangential Flow Filtration System</td>
<td>39</td>
</tr>
<tr>
<td>Figure 21:</td>
<td>3-gauge, 2-valve, Sanitary-fitting Package for a Centrasette C-5 holder</td>
<td>40</td>
</tr>
<tr>
<td>Figure 22:</td>
<td>Tangential Flow Filtration System in Recirculation Mode</td>
<td>45</td>
</tr>
<tr>
<td>Figure 23:</td>
<td>Example of Curve of Concentration versus Absorption (Refractive Index)</td>
<td>48</td>
</tr>
<tr>
<td>Figure 24:</td>
<td>Absorbance Graph</td>
<td>50</td>
</tr>
<tr>
<td>Figure 25:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Cover)</td>
<td>53</td>
</tr>
<tr>
<td>Figure 26:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Compliance Summary)</td>
<td>54</td>
</tr>
<tr>
<td>Figure 27:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Results)</td>
<td>55</td>
</tr>
<tr>
<td>Figure 28:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table I)</td>
<td>56</td>
</tr>
<tr>
<td>Figure 29:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table II)</td>
<td>56</td>
</tr>
<tr>
<td>Figure 30:</td>
<td>Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table III)</td>
<td>57</td>
</tr>
<tr>
<td>Figure 31:</td>
<td>Results of L-929- MEM Cytotoxicity Test (Cover)</td>
<td>58</td>
</tr>
<tr>
<td>Figure 32:</td>
<td>Results of L-929- MEM Cytotoxicity Test (Summary and Compliance)</td>
<td>59</td>
</tr>
<tr>
<td>Figure 33:</td>
<td>Results of L-929- MEM Cytotoxicity Test (Results)</td>
<td>60</td>
</tr>
<tr>
<td>Figure 34:</td>
<td>Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Cover)</td>
<td>61</td>
</tr>
<tr>
<td>Figure 35:</td>
<td>Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Summary and Results)</td>
<td>62</td>
</tr>
<tr>
<td>Figure 36:</td>
<td>Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Verification)</td>
<td>63</td>
</tr>
<tr>
<td>Figure 37:</td>
<td>Endotoxin Levels and Total Oxidizable Substances (Conclusions and Verification)</td>
<td>65</td>
</tr>
</tbody>
</table>
### List of Tables

| Table 1: Identification Code for Membrane Type | 3 |
| Table 3: Identification Codes for Cassette Format and Feed Channel Configuration | 4 |
| Table 2: Identification Codes for Pore Size Rating | 4 |
| Table 4: Identification Codes (if present) for Special Formats | 5 |
| Table 5: Physical Dimensions of Omega Suspended Screen Channel Membrane Cassettes (nominal) | 6 |
| Table 6: Cassette Operating Limits of Pressure, Temperature, and pH | 6 |
| Table 7: Water Flux Specifications For Omega TFF Membranes | 7 |
| Table 8: Solute Passage Specifications | 9 |
| Table 9: Concentration of Solute and Detection Method Used to Test Membranes | 9 |
| Table 10: Membrane Integrity Test — Forward Flow Limits | 10 |
| Table 11: Results of Sodium Hydroxide Compatibility Test | 11 |
| Table 12: Membrane Chemical Compatibility Chart | 12 |
| Table 13: Schedule for Solvent Compatibility Testing | 15 |
| Table 14: Dextran Molecular Weight Fractions and Concentrations in Test Solution | 15 |
| Table 15: Nonvolatile Residue Extracted in Water at 50 ºC from Omega VR Cassettes | 20 |
| Table 16: Results of Cassette Flushing Procedure with Water for Endotoxin Removal | 24 |
| Table 17: Results of Endotoxin Removal for the Challenge Procedure | 25 |
| Table 18: Recommended Torque Values for Manual-torque Cassette Holders | 33 |
| Table 19: Recommended Hydraulic Pressure Range for Pall AT Cassette Holders | 34 |
| Table 20: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes | 35 |
| Table 21: Omega Cassette Hold-up Volumes | 42 |
| Table 22: Relationship of pH to Hydroxyl Ion Molar Concentration | 49 |
| Table 23: Dilution of Control Endotoxin for Standard Curve | 51 |
1 Purpose of this Document

This document provides validation support information for Pall Omega Screen Channel TFF membrane cassettes, and includes summary data to support testing conducted for biological safety, extractables, chemical compatibility, physical and performance attributes, as well as usage conditions (temperature limits, chemical limits, cleaning, flushing, integrity testing, and operating methods).

The data contained in this Guide is generated under standard conditions as specified. The methods and information contained in this Guide are designed to provide the user with an acceptable approach for validation of Omega Screen Channel TFF Membrane Cassettes under actual conditions of use.

Pall Life Sciences offers technical support to customers to develop, troubleshoot, and validate tangential flow filtration procedures.

1.1 Validating Filtration Processes — General Concepts

Tangential flow membrane cassettes play an important role in purifying, concentrating, and separating biopharmaceutical solutions and products. Typical applications include concentrating human plasma fractions, downstream processing of enzyme and protein solutions, and harvesting mammalian or bacterial cells. Hence, the validation of tangential flow filtration processes utilizing membrane cassettes is an essential part of ensuring the manufacture of safe and efficacious products.

The U.S. Food and Drug Administration defines validation as “establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specification and quality attributes” (Current Good Manufacturing Practice (cGMP) for Finished Pharmaceuticals, 21 CFR 210.3). With respect to a tangential flow filtration process, validation involves providing assurance that the filtration process operates reproducibly and consistently.

For any given process, a Functional Design Specification must be written based on the requirements of the process and data generated at the pilot scale. For a tangential flow filtration process utilizing the Omega membrane cassette, this will include developing operational protocols within performance limits outlined in this validation guide and based on the individual cassette operating instructions and care and use manuals supplied with cassettes.

A process system can then be designed and built to allow direct scale-up to meet specifications established at pilot or bench scale. Since tangential flow membrane cassettes are incorporated into complex systems, three stages of system validation are followed: Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ).

1.2 Installation Qualification (IQ)

This checks that the cassettes selected for the process are the ones supplied and installed in the system, and that specified installation procedures such as torque settings have been adhered to. Additionally, it is confirmed that all required documentation has been received (operating instructions, and certificates of conformance) with the cassettes.

1.3 Operational Qualification (OQ)

Engineers test and document the range and operational limits of the filtration process with cassettes in place. Operational qualification (OQ) does not have to be conducted in the customer process manufacturing area. Engineers normally simulate worst-case production conditions for these studies, using water or another surrogate process fluid, to deliberately trigger alarm conditions. As part of the operational qualification, engineers also verify and document procedures such as flushing and sanitizing that are associated with the operation of the membrane cassettes.
1.4 Performance Qualification (PQ)

Performance qualification (PQ) involves testing the cassette filtration process during production of the final product under actual operating conditions, including installation, sanitizing, conditioning, concentration, diafiltration, product recovery, cleaning, etc. Critical elements of performance qualification include verification of chemical compatibility and retention characteristics. Since validating a process ensures the process accomplishes what is intended, performance qualification provides the most meaningful process validation data (which will be confirmed by ongoing performance data collected during system operation) because the data is derived from the process itself, utilizing the intended operating conditions. PQ may not necessarily provide data on the operation of the system at the design limits (alarm conditions), as the process may never reach these limits.

Manufacturers of regulated products must develop and submit protocols, qualification documents, and validation documents for their specific product to be granted approval to manufacture and market their product.
2 Product Specifications

To help you prepare IQ documentation, this section provides you with information on the materials of construction, physical characteristics, and basic performance of Omega membrane cassettes.

2.1 Packaging

Membrane cassettes are individually packaged in heat-sealed plastic bags with the following information printed on the cassette edge (Figure 1):

- Company name
- Membrane type
- Molecular weight cut-off or pore size
- Cassette format
- Membrane area
- Feed channel format
- Part number
- Serial number

Cassettes are shipped in a box containing two silicone gaskets in a plastic bag, Certificate of Test, Membrane Cassettes Care and Use Procedures (R00640 Rev B) and MSDS documents (where appropriate).

Figure 1: Example of Information Printed on the Side of Cassettes

2.2 Part Numbers

Part numbers give specific information about the cassette. For example, the part number OS100C12P1 represents a Omega 100 kDa membrane, 0.01 m$^2$ (0.1 ft$^2$) Centramate™ medium screen channel cassette (Figure 2).

The Part Number for a cassette can be interpreted to identify specific information about the cassette characteristics.

![Part Number Code](image)

Figure 2: Part Number Code

Table 1: Identification Code for Membrane Type

<table>
<thead>
<tr>
<th>Part Number (Digits 1 – 2)</th>
<th>Membrane Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>Omega Membrane Cassette</td>
</tr>
</tbody>
</table>
Table 2: Identification Codes for Pore Size Rating

<table>
<thead>
<tr>
<th>Part Number (Digits 3 – 5)</th>
<th>MWCO or Pore Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>D65</td>
<td>0.65 kDa</td>
</tr>
<tr>
<td>001–500</td>
<td>MWCO in kDa</td>
</tr>
<tr>
<td>990</td>
<td>1000 kDa</td>
</tr>
<tr>
<td>994</td>
<td>0.16 μm</td>
</tr>
</tbody>
</table>

Table 3: Identification Codes for Cassette Format and Feed Channel Configuration

<table>
<thead>
<tr>
<th>Part Number (Digits 6 – 8)</th>
<th>Cassette Format, Feed Channel Configuration (1)</th>
<th>Membrane Area (Nominal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m²</td>
</tr>
<tr>
<td>C10</td>
<td>Centramate™ Fine Screen</td>
<td>0.093</td>
</tr>
<tr>
<td>C11</td>
<td>Centramate Suspended Screen</td>
<td>0.093</td>
</tr>
<tr>
<td>C12</td>
<td>Centramate Medium Screen</td>
<td>0.093</td>
</tr>
<tr>
<td>C05</td>
<td>Centrasette™ Fine Screen</td>
<td>0.46</td>
</tr>
<tr>
<td>C06</td>
<td>Centrasette Medium Screen</td>
<td>0.46</td>
</tr>
<tr>
<td>C07</td>
<td>Centrasette Suspended Screen</td>
<td>0.46</td>
</tr>
<tr>
<td>C20</td>
<td>Centrasette Fine Screen</td>
<td>1.9</td>
</tr>
<tr>
<td>C21</td>
<td>Centrasette Medium Screen</td>
<td>1.9</td>
</tr>
<tr>
<td>C22</td>
<td>Centrasette Suspended Screen</td>
<td>1.9</td>
</tr>
<tr>
<td>C25</td>
<td>Centrasette Fine Screen</td>
<td>2.3</td>
</tr>
<tr>
<td>C26</td>
<td>Centrasette Medium Screen</td>
<td>2.3</td>
</tr>
<tr>
<td>C27</td>
<td>Centrasette Suspended Screen</td>
<td>2.3</td>
</tr>
<tr>
<td>F05</td>
<td>Centrasette II Fine Screen</td>
<td>0.5</td>
</tr>
<tr>
<td>F06</td>
<td>Centrasette II Medium Screen</td>
<td>0.5</td>
</tr>
<tr>
<td>F07</td>
<td>Centrasette II Suspended Screen</td>
<td>0.5</td>
</tr>
<tr>
<td>F20</td>
<td>Centrasette II Fine Screen</td>
<td>2.0</td>
</tr>
<tr>
<td>F21</td>
<td>Centrasette II Medium Screen</td>
<td>2.0</td>
</tr>
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<td>F22</td>
<td>Centrasette II Suspended Screen</td>
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<td>F25</td>
<td>Centrasette II Fine Screen</td>
<td>2.5</td>
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<td>F26</td>
<td>Centrasette II Medium Screen</td>
<td>2.5</td>
</tr>
<tr>
<td>F27</td>
<td>Centrasette II Suspended Screen</td>
<td>2.5</td>
</tr>
<tr>
<td>G01</td>
<td>Maximate™ Medium Screen</td>
<td>0.19</td>
</tr>
<tr>
<td>G02</td>
<td>Maximate Suspended Screen</td>
<td>0.19</td>
</tr>
<tr>
<td>C50</td>
<td>Maxisette™ Fine Screen</td>
<td>2.8</td>
</tr>
<tr>
<td>C51</td>
<td>Maxisette Medium Screen</td>
<td>2.8</td>
</tr>
<tr>
<td>C52</td>
<td>Maxisette Suspended Screen</td>
<td>2.8</td>
</tr>
</tbody>
</table>

(1) All cassettes have fine screens in the filtrate channels. Suspended screen cassettes have fine screens suspended between spacers in the feed channel.
2.3 Serial Numbers

Unique serial numbers enable the tracing of the following cassette information:

- Date of manufacture
- Components used in manufacture
- Water permeability of membrane lot used in construction
- Membrane integrity test results
- Passage and rejection values for specified solutes
- Manufacturing plant location
- Name of the technician who manufactured the cassette

From the serial number and production records, components can be traced back to their source.

2.4 Materials of Construction

2.4.1 Membrane

Omega membranes are cast unsupported from polyethersulfone (PES) resins. Released lots of membrane are laminated on a highly porous polyolefin backing (substrate) that imparts strength and rigidity to the finished membrane. The nominal thickness is 220 μm including the backing.

2.4.2 Screens

Fine screens are constructed of polyester with a nominal thickness of 400 μm. Medium screens are constructed of polyester with a nominal thickness of 565 μm.

2.4.3 Spacers

Omega membrane suspended screen channel cassettes incorporate a screen suspended between spacers in the feed/retentate channel. The spacers are constructed of polyolefin/PES with a nominal thickness of 180 μm.

2.4.4 Encapsulant

The encapsulant is polyurethane.

2.4.5 Gaskets

Gaskets are constructed from USP Class VI medical grade, platinum-cured silicone and have a nominal thickness of 750 μm.

2.5 Dimensions

Pall membrane cassettes are manufactured in a range of formats, and membrane areas (Table 5). This allows the ability to directly scale up or down depending on requirements.
Operating Pressures and Temperatures

### 2.6 Operating Pressures and Temperatures

Membrane cassettes have operating limits for pressure, temperature, and pH (Table 6).

#### Table 6: Cassette Operating Limits of Pressure, Temperature, and pH

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Temperature (°C)</th>
<th>Maximum Recommended Operating Pressure (bar)</th>
<th>pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega Membrane (all formats)</td>
<td>-5 to 50 °C</td>
<td>5 bar (7)</td>
<td>Continuous at 25 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(74 psi)</td>
<td>Cleaning at 45 °C</td>
</tr>
</tbody>
</table>

(5) Freezing can damage cassettes. Additives such as ethanol, glycerin, or salts that depress the freezing point enable the use of cassettes at temperatures below 0 °C.

(6) Maximum pressure limit is 6.8 bar (100 psi) @ 25 °C.

(7) Clamping pressure must be set to the recommended level to avoid leaks.

### 2.7 Water Flux Ranges

Membrane water flux is a measure of the membrane permeability. Water quality, temperature, and pressure affect the water flux rate. At a minimum, the water used to measure flux should be distilled, deionized (DI), 0.2 μm filtered, or preferably, pharmaceutical grade (USP Water for Injection, hereafter called WFI). The presence of biological organisms, organic materials, or minerals in the water may affect water flux results.
2.7.1 Water Flux Specifications for Omega Membranes

Water flux is measured in stirred cells on 43 mm disc membrane samples stamped out from the beginning, middle, and end of each production lot of membrane (Table 7).

Table 7: Water Flux Specifications For Omega TFF Membranes

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Water Flux on Disc Membrane</th>
</tr>
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<tbody>
<tr>
<td>MWCO and Pore Size</td>
<td>mL/min/cm² @ 20 °C</td>
</tr>
<tr>
<td>0.65 kDa</td>
<td>&gt; 0.01 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>1 kDa</td>
<td>&gt; 0.03 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>3 kDa</td>
<td>&gt; 0.08 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>5 kDa</td>
<td>&gt; 0.15 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>10 kDa</td>
<td>&gt; 0.65 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>30 kDa</td>
<td>&gt; 1.5 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>50 kDa</td>
<td>&gt; 1.8 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>70 kDa</td>
<td>&gt; 1.8 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>100 kDa</td>
<td>&gt; 5.0 at 3.74 barg (55 psig)</td>
</tr>
<tr>
<td>200 kDa</td>
<td>&gt; 0.4 at 0.68 barg (10 psig)</td>
</tr>
<tr>
<td>300 kDa</td>
<td>&gt; 0.7 at 0.68 barg (10 psig)</td>
</tr>
<tr>
<td>500 kDa</td>
<td>&gt; 1.0 at 0.68 barg (10 psig)</td>
</tr>
<tr>
<td>1000 kDa</td>
<td>&gt; 1.0 at 0.68 barg (10 psig)</td>
</tr>
<tr>
<td>0.16 μm</td>
<td>&gt; 1.0 at 0.34 barg (5 psig)</td>
</tr>
</tbody>
</table>

2.7.2 Typical Water Flux Values for Omega Membrane Cassettes

Water flux values measured for cassettes are lower than those measured for discs taken from the same lot of membrane (Figure 4: Typical Normalized Water Permeability for Membrane Cassettes compared to a Disc Membrane on page 7). The lower values result from the inherent hydrodynamic resistances within the permeate channel that result in high apparent transmembrane pressure values. Since water permeability is affected by temperature, the measured water fluxes are normalized to 20 °C.

Procedures to determine water flux and normalized water permeability are described in Membrane Cassette Care and Use Procedures.
2.8 Membrane Solute Passage Specifications

Solute passage is determined in stirred cells on 43 mm samples of disc membranes stamped out from the beginning, middle, and end of each production lot of membrane. The process involves adding dilute solute solutions to stirred cells with the membrane installed and performing a 2x concentration. Solute concentration of the total filtrate is determined and compared to initial solute concentration to determine the percent passage. Passage of primary and secondary solutes are measured separately on different membrane discs after determining the water flux for each disc to be within specification (Table 8: Solute Passage Specifications on page 9). Membranes used in cassette construction must meet the solute passage and water flux specifications. Untested samples of membrane are taken every 25 feet from each lot and are retained in case they must be retested at a later date.

Solute passage measurements are not made on finished cassettes because of the invasive nature of the test. However, if needed, you can perform a solute passage test on a cassette to characterize the passage characteristics. The passage data you obtain will vary from disc data because of hydrodynamic differences between the formats; for example, pressure profile and cross flow turbulence. Because the test is invasive, cassettes exposed to a foreign substance would not be reused in many applications (i.e., pharmaceutical processes). For purpose of diagnosing a specific problem, a cassette can be cut open and pieces of membrane removed for testing. Contact Pall for support if this process is required.

The procedure for testing passage in both discs and cassettes is described in Section 8: Method Details on page 43.
### 2.9 Test Solute Concentrations and Detection Methods

Table 9: Concentration of Solute and Detection Method Used to Test Membranes lists the concentration of solutes and the detection method used to test membranes for passage.

<table>
<thead>
<tr>
<th>Test Solute</th>
<th>Source</th>
<th>Concentration (%)</th>
<th>Detection Method</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raffinose</td>
<td>Sigma</td>
<td>0.17</td>
<td>Refractometer</td>
<td>DI water</td>
</tr>
<tr>
<td>Bacitracin B0125</td>
<td>Sigma</td>
<td>0.17</td>
<td>Refractometer</td>
<td>DI water</td>
</tr>
<tr>
<td>PVP K15</td>
<td>ISP Tech.</td>
<td>0.17</td>
<td>Refractometer</td>
<td>DI water</td>
</tr>
<tr>
<td>BSA A7030</td>
<td>Sigma</td>
<td>0.2</td>
<td>UV @ 280 nm</td>
<td>PBS</td>
</tr>
<tr>
<td>IgG G4386</td>
<td>Sigma</td>
<td>0.1</td>
<td>UV @ 280 nm</td>
<td>0.85% w/v in DI water</td>
</tr>
<tr>
<td>Blue dextran D5751</td>
<td>Sigma</td>
<td>0.1</td>
<td>UV @ 550 nm</td>
<td>DI water</td>
</tr>
<tr>
<td>0.14 μm latex bead</td>
<td>Seradyne</td>
<td>0.05</td>
<td>UV @ 550 nm</td>
<td>0.02 – 0.025 g Triton*X100 in 200 mL DI water</td>
</tr>
<tr>
<td>0.21 μm latex bead</td>
<td>Seradyne</td>
<td>0.05</td>
<td>UV @ 650 nm</td>
<td>0.02 – 0.025 g Triton X100 in 200 mL DI water</td>
</tr>
</tbody>
</table>

*Triton is a trademark of Dow Chemical, Inc.

### 2.10 Shelf Life of New and Used Cassettes

The recommended shelf life of cassettes packed in glycerin and sodium azide is five years from date of manufacture. To achieve satisfactory performance, it is recommended that the cassettes be stored unopened in the original packaging at 4 – 25 °C and protected from direct light. For cassettes packed in 0.3 N sodium hydroxide, shelf life is one year with expiration date shown. Users should test the membrane integrity prior to use. Contact Pall for shelf life of cassettes packed in alternate solutions.
The useful life of cassettes that are properly conditioned, used, cleaned, stored, and maintained is often more than one year. However, it is not possible to specify a shelf life or useful life of a cassette that has been used or removed from the original packaging. The actual useful life for a cassette will depend on the character and complexity of the product to which it is exposed, composition of process fluids, process temperatures, operating pressures, and cleaning regime. Therefore, customers should validate reuse and the useful life of a cassette in their process. Pall Life Sciences makes no claims of warranty or guarantee of performance related to reuse of cassettes. Consult Pall Life Sciences’ *Membrane Cassettes Care and Use Procedures* (R00640 Rev B) for recommended storage conditions.

### 2.11 Membrane Integrity—Forward Flow Test Values

The membrane integrity test measures air forward flow rates at specified pressures to determine the integrity of membranes. The air forward flow is a measure of air diffusion through the liquid in the membrane pores, air flow through empty pores, plus air leakage around seals. The test identifies gross defects in the cassette membrane or membrane seals. Membrane integrity Forward Flow test values are given in Table 10: Membrane Integrity Test — Forward Flow Limits for the different cassette formats.

Use only dry filtered air or nitrogen from cylinders (instrument-quality) when using integrity analyzers incorporating mass flow meters. Fluctuations in house air and nitrogen supplies as well as changes in temperature can cause inconsistent results. Fully wet-out the membrane in a cassette prior to performing the membrane integrity test or high forward flow values may be obtained. The procedures for wetting out cassettes and measuring forward flow are described in *Membrane Cassettes Care and Use Procedures* (R00640 Rev B).

### Table 10: Membrane Integrity Test — Forward Flow Limits

<table>
<thead>
<tr>
<th>Membrane MWCO or Pore Size</th>
<th>Test Pressure</th>
<th>Allowable Air Forward Flow Rate per unit area of membrane (9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 kDa and lower MWCO</td>
<td>2.0 bar (30 psi)</td>
<td>≤ 1600 sccm/m² (≤ 150 sccm/ft²)</td>
</tr>
<tr>
<td>1000 kDa and 0.16 μm Omega</td>
<td>0.7 bar (10 psi)</td>
<td>≤ 1076 sccm/m² (≤ 100 sccm/ft²)</td>
</tr>
</tbody>
</table>

(9) Nitrogen can be used in place of air.

### 2.12 Chemical Compatibility—Sodium Hydroxide, 0.05 – 1 N, 20 °C

Sodium hydroxide is the most effective and commonly used agent in biological applications for cleaning, sanitizing, and depyrogenating membrane cassettes. To evaluate the chemical resistance of Omega membrane cassettes, a study was performed in which cassettes were stored at room temperature (20 °C) in different concentrations of sodium hydroxide and periodically tested at intervals for up to one year. A control cassette was stored in water containing 0.05 – 0.1% sodium azide. The following function tests were performed on the cassettes: membrane integrity, water flux, and solute passage.

**Procedure**

Four new Omega 10 kDa Minisette™ screen channel cassettes were removed from the original packaging, placed in a holder, flushed with water, and tested for membrane integrity, water flux, and solute passage. Cassettes met specifications. One cassette was placed in each of the test solutions DI water, 0.1 N, 0.5 N and 1 N NaOH) in covered containers. Cassettes were periodically removed from the storage solution, inserted in holders, flushed with water, and tested for membrane integrity, water flux, and solute passage. They were then cleaned and returned to the appropriate storage container. Final tests were made at 52 weeks.

**Results**

After one year of storage, the samples were within specifications for membrane integrity, water flux, and solute passages. Sodium hydroxide at concentrations up to 1 N showed no significant effect on membrane integrity, water flux, and solute passage characteristics of Omega cassettes stored for a period of one year (Table 11: Results of Sodium Hydroxide Compatibility Test on page 11).
Table 11: Results of Sodium Hydroxide Compatibility Test

<table>
<thead>
<tr>
<th>Chemical</th>
<th>NaOH Concentration (°C)</th>
<th>Temperature (°C)</th>
<th>Exposure Time (weeks)</th>
<th>Integrity Test (10)</th>
<th>Water Flux (11)</th>
<th>Solute Passage (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI Water</td>
<td>—</td>
<td>20</td>
<td>52</td>
<td>pass</td>
<td>pass</td>
<td>pass</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.1 N</td>
<td>20</td>
<td>52</td>
<td>pass</td>
<td>pass</td>
<td>pass</td>
</tr>
<tr>
<td>NaOH</td>
<td>0.5 N</td>
<td>20</td>
<td>52</td>
<td>pass</td>
<td>pass</td>
<td>pass</td>
</tr>
<tr>
<td>NaOH</td>
<td>1.0 N</td>
<td>20</td>
<td>52</td>
<td>pass</td>
<td>pass</td>
<td>pass</td>
</tr>
</tbody>
</table>

(10) Integrity Test—meets specification (less than 25 cc/min/ft² @ 5 psig).
(11) Water Flux—test value is > 80% of initial water flux.
(12) Passage Test—meets specifications for molecular weight cut-off tested.

2.13 Chemical Compatibility — General

Chemical compatibility of membrane cassettes can be described in terms of changes in physical characteristics as a result of continuous contact with a chemical solution for several hours. Changes can affect dimensions, hardness, swelling, integrity of internal seals, and membrane integrity. Changes can also be described in terms of functional characteristics of the membrane (such as water permeability, retention characteristics).

Table 12 illustrates the chemical compatibility of Omega membrane cassettes at 20 °C (unless otherwise noted) with respect to physical characteristics. Table 12 should be used only as a guide. Cassettes should be tested in the appropriate solvent and product under actual operating conditions and for an appropriate time to determine compatibility for the specific application. Membrane porosity— and consequently both water permeability and retention characteristics —may be affected. Physical changes to the cassette may be permanent or reversible. To determine if changes are permanent, flush and then soak the cassette in water for one to two days and then test the sample again. Changes in water permeability and solute retention may be due to physical changes in the membrane. Changes in solute retention may be a result of conformational changes in the molecules used to measure retention, or a combination of factors.
2.14 Protein Binding Characteristics

Omega membranes have low nonspecific adsorption characteristics. The actual amount of protein or other substances that will adsorb (nonspecifically bind) to the membrane is dependent on the specific characteristics of that substance. Adsorption of a molecule to a membrane can vary to a great extent depending on its environment (i.e., the chemical composition of the solution it is in, as well as the number and concentration of other solutes present). Changes in pH, ionic strength, temperature and concentration have a significant effect on binding properties. Buffer salts, detergents, and organic solvents also influence binding. If adsorption is a concern, then a study can be performed on a disc membrane using the actual sample and buffer to determine the level of nonspecific adsorption.

Figure 5: Nonspecific Adsorption Characteristics of Three Membrane Materials on page 13 compares the nonspecific adsorption characteristics of three different membrane materials for albumin and cytochrome C in phosphate-buffered saline at pH 7.0. The isoelectric points of albumin and cytochrome C are 4.6 and 10 respectively. Therefore, at pH 7, albumin is negatively charged and cytochrome C is positively charged.

The nonspecific adsorption characteristics of Omega membrane is similar to regenerated cellulose and significantly lower than unmodified polyethersulfone.
Figure 5: Nonspecific Adsorption Characteristics of Three Membrane Materials
3 Validation Procedures

This section describes the procedures Pall used to validate specific chemical and physical characteristics of the Omega membrane cassettes.

3.1 Solvent Compatibility

3.1.1 Introduction

Cassettes may be exposed to a number of solvents, primarily during sanitization and cleaning procedures. To evaluate the effect of commonly used solvents on cassette performance, cassettes were exposed to solvents under conditions that simulated typical worst case use. The criteria used to evaluate the performance of the cassettes after exposure to solvents were water permeability, membrane integrity test, physical appearance, and hardness of the urethane encapsulant.

3.1.2 Scope

Four solutions were chosen as representative of commonly used cleaning agents. The four solutions and exposure conditions were:

- 0.5 N NaOH at 45 °C for 28 days
- 0.5 N NaOH + 400 ppm NaOCl at 45 °C for 28 days
- 40% EtOH at 23 °C for 28 days
- 0.1 N HNO₃ at 45 °C for 14 days

A typical cleaning regime is less than three hours. Cleaning with acids such as nitric acid is used less frequently, for shorter periods (1 – 1.5 hours) and often in addition to a caustic cleaning. Therefore, the exposure time in this study is equivalent to one cleaning cycle/day (5 days per week) for one year.

Omega 10 kDa membrane Centrasette screen channel cassettes 0.5 m² (5 ft²) were chosen for this study. The materials of composition and construction of Omega cassettes are the same; hence, they should be similarly affected by the test solutions. A 10 kDa molecular weight cut-off was chosen because solvents should have a greater effect on the porosity of a tight membrane compared to a membrane with larger pores. The work was performed in duplicate.

3.1.3 Summary of Method

Cassette Preparation

1. Assemble a Centrasette C-5 system.
2. Install the Omega 10 kDa membrane Centrasette cassette.
3. Flush and sanitize the cassette according to the Membrane Cassettes Care and Use Procedures (R00640 Rev B).
4. Determine water flux with a 2 barg (30 psig) feed pressure and a 0.7 barg (10 psig) retentate pressure (TMP = 1.35 barg (20 psig)) at 20 – 23 °C. Record the results.
5. Perform the system pressure hold and membrane integrity tests and record results.
6. Flush the cassette with water, 50 L/m² (5 L/ft²) through the retentate port then 50 L/m² (5 L/ft²) through the filtrate port.
7. Pump air through the cassette to expel the water.
8. Recirculate the test solution through the cassette for 10 minutes. Use a cross flow of about 5 – 7 L/min/m² (0.5 – 0.7 L/min/ft²). Then, remove the cassette from the holder and place in a covered container filled with the test solution and maintained at the specified temperature.
9. Test the cassette 2 – 3 times a week (Table 13: Schedule for Solvent Compatibility Testing).
Table 13: Schedule for Solvent Compatibility Testing

<table>
<thead>
<tr>
<th>Solution</th>
<th>Temperature (°C)</th>
<th>Test Period (days)</th>
<th>Test Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 N NaOH</td>
<td>45</td>
<td>28</td>
<td>2 to 3 times per week</td>
</tr>
<tr>
<td>0.5 N NaOH, 400 ppm NaOCl</td>
<td>45</td>
<td>28</td>
<td>2 to 3 times per week</td>
</tr>
<tr>
<td>40% EtOH</td>
<td>23</td>
<td>28</td>
<td>2 to 3 times per week</td>
</tr>
<tr>
<td>0.1 N HNO₃₃</td>
<td>45</td>
<td>14</td>
<td>2 to 3 times per week</td>
</tr>
</tbody>
</table>

Cassette Evaluation Procedure

1. Remove the cassette from the storage container.
2. Drain and rinse the cassette with water. Observe the appearance of the cassettes. Measure hardness. Note any change to physical characteristics (i.e., color, swelling).
3. Install the cassette in the holder using proper torque values.
4. Flush the cassette with water 50 L/m² (5 L/ft²) through the retentate port then 50 L/m² (5 L/ft²) through the filtrate port. Set the system for total recirculation and run for 5 minutes. Adjust the feed pressure to 2 barg (30 psig) and the retentate pressure to 0.7 barg (10 psig).
5. Perform the pressure hold and membrane integrity tests as detailed in the Membrane Cassette Care and Use Procedures.
6. Set the system for total recirculation with water and run for 5 minutes. Adjust the feed pressure to 2 barg (30 psig) and retentate pressure to 0.7 barg (10 psig). Run until the air is expelled from the cassette.
7. Determine water flux with a 2 barg (30 psig) feed pressure and a 0.7 barg (10 psig) retentate. Record the results.
8. Pump air through the cassette to expel the water.
9. Fill the cassette by recirculating the test solution through it. Then remove the cassette from the holder for continued static soak.
10. End testing when the membrane integrity test fails; or if the water flux increases or decreases to 2 times the original value; or the end of the test schedule is reached.

Results
No changes in appearance were noted in any of the cassettes during the study. The urethane encapsulant showed no significant change in hardness. System pressure hold and membrane integrity values were well below the specification limit. Water flux decreased slightly (< 15%) for both acid and basic solutions. The ethanol solution showed a slight increase in water flux value (Table 14: Results of Solvent Compatibility Test for Omega Membranes).

Table 14: Results of Solvent Compatibility Test for Omega Membranes

<table>
<thead>
<tr>
<th>Solution</th>
<th>Test Period (days)</th>
<th>Initial Water Flux (LMH/psig)</th>
<th>Water Flux at End (LMH/psig)</th>
<th>% of Initial Flux</th>
<th>Membrane Integrity (initial) (mL/min/ft²)</th>
<th>Membrane Integrity (final) (mL/min/ft²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 N NaOH, 45 °C</td>
<td>28</td>
<td>11</td>
<td>10</td>
<td>90</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>0.5 N NaOH, 400 ppm NaOCl 45 °C</td>
<td>28</td>
<td>12</td>
<td>11</td>
<td>92</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>40% EtOH, 23 °C</td>
<td>28</td>
<td>13</td>
<td>14</td>
<td>108</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>0.1 N HNO₃₃, 45 °C</td>
<td>14</td>
<td>12</td>
<td>11</td>
<td>92</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Conclusion
Omega membrane cassettes are compatible with the tested cleaning solutions at the temperature and the duration evaluated in this study.
3.2 Retention Characteristics — Dextran Selectivity Procedure

Introduction
Ultrafiltration membranes are tested for their retention properties by challenging them with various sized molecules and measuring the percentage retained (or passed) by the membrane. The mixed dextran procedure challenges the membrane with a solution containing a heterogeneous mixture of molecules of different molecular weights. Thus, selectivity curves that characterize the behavior of the membrane can be generated by comparing the distribution of molecules upstream of the membrane to the distribution in the permeate (Figure 6: Example of Dextran Selectivity Curves for Omega Membranes on page 16). Dextrans of different molecular weights may be mixed together in a solution without concern of interactions between the various molecules.

Dextran is a predominantly linear polymer consisting of repeating units of glucose monomer, and it was selected because of its low binding characteristics and availability. The mixed dextran test uses dextran fractions with molecular weight ranging from 1,000 to 2,000,000 daltons. Normally, dextrans show higher passage than globular proteins of similar molecular weight. Thus, you cannot use the dextran selectivity curve to determine the molecular weight cut-off of a membrane. However, the data is indicative of the pore size distribution of the membrane. You can use a mixed dextran analysis to compare selectivity between different lots of membrane, to confirm selectivity has not changed, or to validate that the molecular weight selectivity of a membrane is as represented.

![Figure 6: Example of Dextran Selectivity Curves for Omega Membranes](image)

The results of a mixed dextran challenge are dependent on the process conditions. An accurate comparison can only be made when selectivity curves are determined using the same test conditions. Changes in dextran concentration, cross flow rates, transmembrane pressure or temperature may significantly change the results. To minimize the effect of gel polarization, dextran solutions are prepared at very low concentrations and the transmembrane pressure is maintained at about 0.07 – 0.14 barg (1 – 2 psig) by controlling the filtrate flux rate with the filtrate valve.

You can use the following procedure to determine dextran selectivity curves for Omega cassettes.

3.2.1 Summary of Method

**Equipment**
- Hewlett Packard 1050 HPLC equipped with a refractive index detector
- TSKG4000PW gel filtration column (Toso Haas)
- Dextran standards and fractions (Fluka)

**Preparation of Dextran Challenge Solutions**
Prepare the dextran challenge solution at a volume of 0.5 L of challenge solution per 0.09 m² (1 ft²) of membrane at concentrations to give approximately equal responses on the refractive index detector (Table 15).
Calibration of the HPLC Column
Prepare samples of each of the dextran standards at concentrations of 0.50 g/L in DI ultrafiltered (through a 10 kDa membrane) water. Chromatographically analyze each standard on the column. Plot the elution time of the resulting peak for each standard against its molecular weight to create a standard curve (Table 15: Dextran Molecular Weight Fractions and Concentrations in Test Solution). This curve will be used to generate selectivity profiles for subsequent tests.

**Table 15: Dextran Molecular Weight Fractions and Concentrations in Test Solution**

<table>
<thead>
<tr>
<th>Molecular Weight (kDa)</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.80</td>
</tr>
<tr>
<td>6</td>
<td>0.70</td>
</tr>
<tr>
<td>20</td>
<td>0.65</td>
</tr>
<tr>
<td>40</td>
<td>0.60</td>
</tr>
<tr>
<td>70</td>
<td>0.50</td>
</tr>
<tr>
<td>110</td>
<td>0.55</td>
</tr>
<tr>
<td>220</td>
<td>0.60</td>
</tr>
<tr>
<td>550</td>
<td>1.1</td>
</tr>
<tr>
<td>2000</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Membrane Cassette Test Procedure
Set up a tangential flow filtration system with a cassette installed in the holder according to the manufacturer’s instructions. Flush the cassette with water. (Recommended flushing procedure and cross flow rates are listed in the Membrane Cassette Care and Use Procedures). Drain the feed reservoir and add the dextran challenge solution to the reservoir. Set the system up to run in recirculation mode (Figure 22: Tangential Flow Filtration System in Recirculation Mode on page 45) with the filtrate valve closed. Recirculate the dextran solution at the recommended cross flow rate. Adjust the retentate valve to produce a retentate pressure of 0.07 – 0.14 barg (1 – 2 psig). Slowly open the filtrate valve just enough to allow a permeate rate of about 6 – 7 LMH. After 15 minutes, take a feed and filtrate sample. Inject the samples on the TSKG4000PW column and record the chromatograms (Figure 8).
Data and Calculations

Tabulate the refractive index detector response, with the background subtracted, for both the feed and filtrate chromatograms at the predetermined time interval. After tabulating the detector responses at the selected time intervals, find the membrane percent rejection for each time increment using the following equations:

\[
\text{Rejection} = 1 - \left( \frac{\text{filtrate response}}{\text{feed response}} \right)
\]

\[
\% \text{ rejection} = \text{rejection} \times 100
\]

The data at this point is percent rejection by membrane versus time. The second step of the process is to convert retention time to molecular weight using a column calibration curve (Figure 7: Molecular Weight of Dextran Versus Retention Time on TSK G4000PW Column on page 17). Plot the percent rejection against the molecular weight of the dextrans on a semilog plot to produce a selectivity curve (Figure 9: Example of Membrane Percent Rejection versus Dextran Molecular Weight Curve on page 18).


Conclusion

The mixed dextran challenge is an efficient and accurate way of determining the selectivity characteristics of an ultrafiltration membrane. This test provides the user of ultrafiltration membranes with a nondestructive method to compare and evaluate the selectivity of a new membrane as it compares to previous membranes. It also allows comparison of membranes with different characteristics or from different manufacturers. This information may help in determining the suitability of a membrane for a specific application. A membrane’s molecular weight cut-off is
determined using specific test solutes; not dextran. The dextran challenge cannot be used as a substitute to determine molecular weight cut-off.

### 3.3 Extractables Test

#### 3.3.1 Introduction

The purpose of this test is to quantify and characterize the nonvolatile materials that may be extracted from Omega membrane cassettes into aqueous products. The extracted material was analyzed by infrared (FTIR) and ultraviolet spectrophotometry. The cassettes tested contained approximately 0.19 m² (2.0 ft²) of a polyethersulfone filter membrane interleaved between polyester screen spacers. The repeating layers of membrane and screen spacer are encapsulated with a polyurethane sealant. Centramate, Centrasette, Maximate and Maxisette cassettes are constructed from the same raw materials and by the same procedure. Therefore the type of extractables determined for one format should be the same for all formats.

#### 3.3.2 Summary of Method

Omega 300 kDa VR membrane, Maximate screen channel cassettes from inventory, catalog number OS300G01VR, were tested for extractable material. The cassettes were flushed and sanitized following protocols generally outlined in *Membrane Cassette Care and Use Procedures*. The test cassettes were then subjected to a rigorous extraction procedure by recirculating either water at 45 – 55 °C or 25% ethanol at 20 – 25 °C for 16 hours according to the extraction procedure described in Section 8: Method Details on page 43. After this extraction period, the fluid was drained and collected from the system for analysis. A second 16-hour extraction of each cassette (with the same solvent and conditions) followed by recovery of the extraction fluid was then performed to demonstrate presence of additional extractables. These test conditions were chosen to maximize the amount of extracted matter. A negative control (holder and cut-out gasket only, no cassette) and positive control (known amount of a known substance) were performed for each test solvent. Collected samples and controls were analyzed by ultraviolet/visible absorption spectroscopy over the range 200 – 360 nm. A 1,000 mL aliquot of each extract was evaporated to dryness and the amount of nonvolatile residue was weighed and characterized by infrared spectroscopy. Details of the test methodology are provided in Section 8.

#### 3.3.3 Results

After completing the recommended procedures for sanitizing, flushing, and integrity testing, the Omega VR membrane Maximate™ screen channel cassettes tested produced 255 – 527 mg of nonvolatile residue when extracted with water at 50 °C and 413 – 520 mg of nonvolatile residue when extracted with 25% ethanol at ambient temperature (20 – 25 °C). Successive extractions of each cassette produced decreasing amounts of nonvolatile residue. The total of the first and second extractions with water for the cassette with the greater nonvolatile residue (S/N 37234277) was 527 mg. The total of the two extractions with 25% ethanol for the cassette with the greater nonvolatile residue (S/N 37234276) was 520 mg (Table 16: Nonvolatile Residue Extracted in Water at 50 °C from Omega VR Cassettes and Table 17: Nonvolatile Residue Extracted in 25% Ethanol at 20 – 25 °C from Omega VR Cassettes).

The ultraviolet absorption spectra of the extracts were measured using a Hewlett-Packard Model 8452A diode array spectrophotometer. The extracts only had measurable UV absorbance below 220 nm (0.25 to 0.95 OD at 200 nm), and no UV absorbance maximum was measured over the range of 200 to 360 nm. The infrared spectra of the nonvolatile residues were measured using samples in the form of potassium bromide pellets in a Nicolet Model 510P Fourier Transform infrared spectrophotometer.

The infrared absorption spectrum indicates that the material consists mainly of glycerin, which is used as a storage solution. An additional signal at 2039 – 2044 cm⁻¹ in the cassette extracts, which is absent in a reference spectrum of glycerin, can be attributed to trace amounts of stabilizers or decomposition or polymerization by-products from the polyurethane. Minor signals at 1550 – 1720 cm⁻¹, typical of carbonyl moieties, may represent traces of oligomers from the polyurethane sealant used to encapsulate the membrane (Figure 10: Infrared Spectrum of Nonvolatile Residue (Water Extract) as Smear on KBr on page 20 and Figure 11: Infrared Spectrum of Nonvolatile Residue (25% Ethanol Extract) as Smear on KBr on page 20). Additional testing by flame spectrochemical analysis of NVR from cassettes pre-washed in water alone showed sodium to be below the detection limit confirming that sodium azide was not detectable.
### 3.3.4 Conclusions

The aqueous extractables found for Omega VR membrane Maximate cassettes using both water and 25% ethanol in water are comprised primarily of glycerin, which is the storage solution residue. This was determined by comparing the IR spectral results with standard spectra of suspected extractables.

![Figure 10: Infrared Spectrum of Nonvolatile Residue (Water Extract) as Smear on KBr](image1)

![Figure 11: Infrared Spectrum of Nonvolatile Residue (25% Ethanol Extract) as Smear on KBr](image2)

<table>
<thead>
<tr>
<th>Cassette S/N</th>
<th>First Extraction Nonvolatile Residue (mg/2 L)</th>
<th>Second Extraction Nonvolatile Residue (mg/2 L)</th>
<th>Sum of First and Second Extractions (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37234275</td>
<td>214</td>
<td>41</td>
<td>255</td>
</tr>
<tr>
<td>37234277</td>
<td>508</td>
<td>19</td>
<td>527</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cassette S/N</th>
<th>First Extraction Nonvolatile Residue (mg/2 L)</th>
<th>Second Extraction Nonvolatile Residue (mg/2 L)</th>
<th>Sum of First and Second Extractions (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37234276</td>
<td>336</td>
<td>184</td>
<td>520</td>
</tr>
<tr>
<td>37234278</td>
<td>276</td>
<td>137</td>
<td>413</td>
</tr>
</tbody>
</table>

### 3.4 Cassette Flushing Procedures to Remove Storage Agents

Omega membrane cassettes are shipped wet, in liquid containing a humectant and bactericidal storage solution. This solution consists of DI water containing approximately 15 – 20% glycerin, which serves to prevent the membranes
from drying out, and 0.05 – 0.1% sodium azide, a bactericidal agent. The storage solution must be removed and the cassette flushed well with water prior to use to prevent product contamination. The purpose of this study was to determine the effectiveness of recommended flushing procedures for removal of these agents.

3.4.1 Sanitization and Flush Procedure for Omega Membrane Cassettes

The procedure recommended for flushing these agents out of the cassette prior to use is described in *Membrane Cassette Care and Use Procedures*.

3.4.2 Summary of Method

To evaluate the effectiveness of this procedure for removal of the storage solution from Omega, suspended screen channel membrane cassettes, a test was conducted using the following procedure:

1. A Maximate cassette with Omega 300 kDa VR membrane was installed in a cassette holder.
2. The system was flushed with sanitizing solution (0.1 N NaOH) by recirculating the solution 30 – 45 minutes.
3. The sanitizing solution was replaced with DI water.
4. With filtrate valve closed, at least 55 liters of water per m² (5 L/ft²) of installed membrane area was flushed through the retentate line to waste. Samples were taken at every 0.5 – 1.0 liters.
5. The retentate valve was closed and filtrate valve was opened. At least 55 liters of water per m² (5 L/ft²) was flushed through the filtrate line to waste. Samples were taken every 0.5 – 1.0 liters.
6. The glycerin concentration of both feed and filtrate samples was determined using a size exclusion chromatography method.
7. The sodium azide concentration of both feed and filtrate samples was determined using a spectrophotometric method.

Details of the test methodology are provided in Section 9.2: Detection Methods for Storage Agents on page 49.

3.4.3 Results

The concentration of residual glycerin in the filtrate and retentate samples was reduced to less than 10 ppm after 5 liters of flushing. The concentration of residual sodium azide in the filtrate and retentate samples was reduced to less than 1 ppm after 5 liters of flushing (Figure 12). The limit of detection in both analytical methods was 1 ppm.

3.4.4 Conclusions

The concentration levels of glycerin and sodium azide decreased rapidly from both the retentate and filtrate channels of Omega 300 K VR membrane Maximate cassettes with DI water flushing.
3.5 Cassette Flushing Procedure — Low Volume, Multiple Recirculation

3.5.1 Introduction
Reducing the residual glycerin concentration below 10 ppm and sodium azide concentration below 1 ppm by continuous flushing requires large volumes of water. The same results can be achieved with much smaller volumes of water. These limits can be reached by an initial flushing to drain followed by recirculating clean water for approximately one hour followed by another flushing with clean water. This process may be repeated several times until the glycerin and sodium azide concentrations have been reduced to acceptable levels.

3.5.2 Procedure
These steps were followed to demonstrate the effectiveness of the low-volume, multiple recirculation flushing process:

1. A TFF system including a Centrasette C-5 holder was prepared. An Omega 10 kDa membrane Centrasette screen channel cassette was installed in the holder and torqued to specifications.
2. The filtrate valve was closed. The retentate valve was opened completely. 20 L/m² (2 L/ft²) of water was added to the feed reservoir.
3. 10 L/m² (1 L/ft²) of water was flushed to the drain.
4. The filtrate valve was opened. The retentate valve was closed almost completely. The remaining water was flushed to the drain.
5. The system was set up for recirculation with both retentate and filtrate valves open.
6. 10 L/m² (1 L/ft²) of water was added to the feed reservoir. The pump was adjusted to deliver a volumetric flow rate equal to recommended process cross flow for cassette type.
7. The retentate valve was adjusted so that filtrate flow rate was ~½ the retentate flow rate. Retentate flow rate was readjusted. Feed pressure was not allowed to exceed 2 barg (30 psig).
8. The solution was recirculated for one hour and then a sample of the feed was taken.
9. The retentate and filtrate valves were opened completely. The retentate and filtrate lines were directed to waste and system was pumped dry.
10. L/m² (1 L/ft²) of water was added to the feed reservoir. The filtrate valve was closed. 5 L/m² (0.5 L/ft²) was pumped to waste. The filtrate valve was opened and the retentate valve was closed. The remaining water was pumped through the filtrate line to waste.
11. If needed, steps 5 – 10 were repeated until glycerin and sodium azide concentrations of the feed sample were acceptable.

3.5.3 Results
Figure 13 illustrates the results from a study done on an Omega 10 kDa membrane Centrasette screen channel cassette. Zero cycles defines the starting concentration of glycerin and sodium azide of the storage solution in the cassettes. Each additional cycle gives the concentration of extracted glycerin in recirculated water after one hour. At 5 cycles, the concentration of extracted glycerin from the cassette in water was less than 10 ppm in 10 L/m².
Validation Procedures

(1 L/ft²) per membrane area. Sodium azide was below the detection limit (0.1 ppm). Each additional cycle reduced the extractable glycerin concentration further.

Figure 13: Results of Low Volume, Multiple Recirculation Flushing to Remove Sodium Azide and Glycerin

3.5.4 Conclusion
The concentration of glycerin extracted from the cassette after five cycles is less than 10 ppm in 10 L/m² (1 L/ft²) of water per membrane area. Sodium azide concentration was below the detectable limit (0.1 ppm). Since typical process volume-to-membrane area ratios are substantially (>10 times) greater, the level of glycerin that may be present as a contaminant should be below 1 ppm. Additional extraction cycles with clean water reduces the level of extractable glycerin further. Using five extractions requires approximately 80 – 100 L/m² (8 – 10 L/ft²) of water per membrane area and takes about six hours.

The glycerin concentration measured by this procedure is a combined extraction from both the feed and filtrate. The actual concentration in the feed alone will be much lower.

3.6 Sanitization — Endotoxin Removal — New Cassette Flush Procedure

Endotoxin — high molecular weight lipopolysaccharides — is associated with the cell wall of gram negative bacteria. As part of normal functioning and during cell autolysis, bacteria release endotoxins into their environment. Unpurified endotoxin contains lipids, carbohydrates, and protein. Aggregated forms of endotoxin have molecular weights ranging from about 300,000 to 1,000,000 daltons. Active subunits can occur with molecular weights as small as ten thousand daltons. Endotoxin is pyrogenic, that is, it can cause fever in humans and animals, so it is imperative that pharmaceutical products, especially injectables, are pyrogen-free. Endotoxin can be detected by the Limulus Amebocyte Lysate (LAL) procedure. The lower detection limit is based on the method and sensitivity of the lysate being used.

1 EU (endotoxin unit) = 100 picograms endotoxin

3.6.1 Introduction
Cassettes are not supplied sterile, and Pall does not claim that cassettes are endotoxin-free. Therefore, they should be flushed and sanitized prior to use. To evaluate initial endotoxin levels and the effectiveness of the recommended flushing procedure, the following study was performed.

3.6.2 Summary of Method
Two new Omega. 10 kDa membrane Centrasette screen channel cassettes (part number OS010C05) and two new suspended screen channel cassettes (part number OS010C07) were taken from inventory and tested separately using the procedure below. Endotoxin levels were determined according to the procedure for LAL testing using the ThermoMax assay. The LAL procedure is described in Section 8: Method Details on page 43.
1. Cassettes were installed in a Centrasette C-5 holder and system that had been previously cleaned and tested to be free of endotoxin.
2. Endotoxin-free water (≤ 0.01 EU/mL), 200 L/m² (20 L/ft²), was flushed through the cassette and out the retentate to waste (filtrate closed). Samples were collected at beginning and end of flush.
3. Endotoxin-free water (≤ 0.01 EU/mL), 40 L/m² (4 L/ft²), was flushed through the membrane and out the filtrate to waste (retentate closed). Samples were collected at beginning and end of flush.
4. The system was then drained and set up for recycle. Two liters of pyrogen-free water were added and recirculated for one hour. Samples of both the retentate and filtrate were taken after one hour.
5. Because the cassettes were to be used for further testing, they were flushed with 0.1 N NaOH, removed from the holder, and stored in a covered container containing 0.1 N NaOH.

3.6.3 Results
The endotoxin level in water first flushed from new cassettes was measured at less than or equal to 0.2 EU/mL. Flushing the cassette with pyrogen-free water — 200 L/m² (20 L/ft²) through the retentate and 40 L/m² (4 L/ft²) through the filtrate — reduced the endotoxin level below 0.01 EU/mL. Recirculating 2 – 4 L/m² (0.2 – 0.4 L/ft²) of endotoxin-free water through the cassette for one hour resulted in endotoxin levels of ≤ 0.02 EU/mL (Table 18).

3.6.4 Conclusions
The endotoxin level in water flushed from a new Omega membrane Centrasette cassette is very low. Flushing to waste — 200 L/m² (20 L/ft²) through the retentate and 40 L/m² (4 L/ft²) through the filtrate — with endotoxin-free water reduced the endotoxin level below 0.01 EU/mL in the effluent.

Table 18: Results of Cassette Flushing Procedure with Water for Endotoxin Removal

<table>
<thead>
<tr>
<th>Omega 10 kDa Endotoxin Level EU/mL</th>
<th>Screen Channel Cassette</th>
<th>Omega 10 kDa</th>
<th>Lot # 38058017</th>
<th>Omega 10 kDa</th>
<th>Lot # 38058017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>0.020 @ 5 L</td>
<td>0.121 @ 1 L</td>
<td>0.087 @ 1 L</td>
<td>0.037 @ 5 L</td>
<td></td>
</tr>
<tr>
<td>End of flush</td>
<td>&lt; 0.01 @ 100 L</td>
<td>&lt; 0.01 @ 20 L</td>
<td>&lt; 0.01 @ 100 L</td>
<td>&lt; 0.01 @ 20 L</td>
<td></td>
</tr>
<tr>
<td>Module recirculation after 1 hour</td>
<td>0.060</td>
<td>&lt; 0.01</td>
<td>0.013</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Omega 10 kDa Endotoxin Level EU/mL</th>
<th>Suspended Screen Channel Cassette</th>
<th>Omega 10 kDa</th>
<th>Lot # 38111018</th>
<th>Omega 10 kDa</th>
<th>Lot # 38111019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>0.014 @ 5 L</td>
<td>&lt; 0.01 @ 1 L</td>
<td>&lt; 0.01 @ 5 L</td>
<td>&lt; 0.01 @ 1 L</td>
<td></td>
</tr>
<tr>
<td>End of flush</td>
<td>&lt; 0.01 @ 100 L</td>
<td>&lt; 0.01 @ 20 L</td>
<td>&lt; 0.01 @ 100 L</td>
<td>&lt; 0.01 @ 20 L</td>
<td></td>
</tr>
<tr>
<td>Module recirculation after 1 hour</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

(14) Measured endotoxin level in endotoxin-free water was < 0.01 EU/mL.

3.7 Sanitization — Endotoxin Challenge Procedure

3.7.1 Introduction
Membrane cassettes are typically cleaned and reused many times. There is a risk of contamination with endotoxin from previous samples, or from bacterial growth during storage. Therefore, you should remove or reduce endotoxin levels in the cassette prior to use with a new sample. The following study was performed to show the effectiveness of the flushing/depyrogenation procedure recommended in Membrane Cassette Care and Use Procedures.

3.7.2 Summary of Method
Cassettes from the previous flushing study, Section 3.6: Sanitization — Endotoxin Removal — New Cassette Flush Procedure on page 23, were installed in a Centrasette C-5 holder. The filtration system was flushed to waste with...
endotoxin-free water to remove the storage solution. The system was then drained. The filtration system was set for recirculation — 85% through retentate and 15% through filtrate. Cassettes were then challenged by adding two liters of a solution containing 1,000 EU/mL and recirculating the solution for one hour through the cassettes. Following recirculation with the challenge solution, the cassettes were flushed with water to waste — 80 L/m² (8 L/ft²) through retentate, 40 L/m² (4 L/ft²) through filtrate. Samples of filtrate and retentate were taken at the end of the flush. The cassettes were then sanitized with 0.1 N NaOH, drained, and flushed with endotoxin-free water. Samples of effluent from both the retentate and filtrate were taken at the end of each NaOH flush. The filtration system was set up for recirculation. Two liters of endotoxin-free water were added and recirculated through both retentate (85%) and filtrate (15%) for one hour. Samples of both retentate and filtrate were taken after one hour. Endotoxin levels were measured on the samples.

3.7.3 Results
Flushing the Centrasette cassettes with water — 80 L/m² (8 L/ft²) through retentate, 40 L/m² (4 L/ft²) through filtrate — following the endotoxin challenge resulted in endotoxin levels below 0.05 EU/mL in the flushing solution. The endotoxin level in the flushing solution after NaOH sanitization was less than or equal to 0.01 EU/mL (same as influent). The endotoxin level in the water (2 L) recirculated for one hour was less than 0.02 EU/mL (Table 19: Results of Endotoxin Removal for the Challenge Procedure).

Table 19: Results of Endotoxin Removal for the Challenge Procedure

<table>
<thead>
<tr>
<th>Sources of Sample</th>
<th>Retentate</th>
<th>Filtrate</th>
<th>Retentate</th>
<th>Filtrate</th>
<th>Retentate</th>
<th>Filtrate</th>
<th>Retentate</th>
<th>Filtrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge solution, prepared to reach 1,000 EU/mL</td>
<td>986.1</td>
<td>1195</td>
<td>1174</td>
<td>1150</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post challenge flush 40 L — retentate 20 L — filtrate</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>0.117</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Flush after 1st NaOH, 40 L — retentate 20 L — filtrate</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Flush after 2nd NaOH, 40 L — retentate 20 L — filtrate</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>2 L recirculation with DI Water</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.14</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DI Water</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.7.4 Conclusions
The flushing procedure recommended by Pall Life Sciences (as described in Membrane Cassette Care and Use Procedures) effectively lowers endotoxin levels. Using the flushing procedure on a previously contaminated cassette reduced the endotoxin level in the final flush to less than or equal to 0.01 EU/mL, a level equal to the measured level in the endotoxin-free water used to flush the cassettes.
4 Quality Assurance (QA)

Membranes and cassettes are produced in conformance with the Pall Corporation manufacturing documentation. Cassette components meet current standards for USP Class VI, 70 °C Biological Reactivity Tests for Plastics.

4.1 Quality Control Measures

Raw materials used in production are logged in for traceability and quarantined upon receipt. After inspection by the Quality Control Department, approved raw materials are issued to the warehouse for storage.

During manufacturing, multiple samples from the beginning, middle, and end of each lot of membrane are tested for quality. Tests include water permeability and the retention/passage characteristics of selected solute molecules applicable to the specific molecular weight cut-off or pore size of the membrane. As required, membrane is removed from inventory to the assembly area and die cut into sheets of the required format. Each die cut sheet is visually inspected for defects. Cassettes are produced according to an assembly procedure from approved lots of membrane and other raw materials, which are recorded on a lot control card. The finished cassette is visually inspected, stamped with the cassette identification, and released from assembly to quality control with the accompanying lot card.

Quality control inspects each cassette and lot card for completeness. The cassettes are then flushed with a glycerin/sodium azide solution and membrane integrity is tested.
4.1.1 Quality Assurance Certificates

A quality assurance certificate is packaged with each tangential flow cassette (Figure 14).

![Certificate of Test](image)

Figure 14: Example of a Certificate of Test
4.1.2 Labels
Each cassette, sealed in a plastic bag, is inserted into a cardboard cradle, and then packaged in a box. Labels affixed to the cradle, box and bag describe the contents (Figure 15: Example of Box Label on page 28). The label identifies the cassette format and contains the part number, and the serial number. This information should match the information printed on the side of the cassette.

![Figure 15: Example of Box Label](image)

4.1.3 Cassette
The following information is printed on the side of each cassette (Figure 16):

1. Company name
2. Membrane type
3. Molecular weight cut-off or pore size
4. Cassette format
5. Membrane area
6. Feed channel format
7. Part number
8. Serial number (formerly lot number)

![Figure 16: Example of the Information Printed on Omega Cassettes](image)

4.2 Drug Master Files
A Drug Master File is a document on file with the FDA. Manufacturers, such as Pall Corporation, create a Drug Master File portfolio and file it with the FDA. The Drug Master File portfolio describes what is involved in the manufacturing of the product — a membrane cassette for example. The FDA assigns a number to each submission. An applicant (end user) for an Investigational New Drug (IND), Product License Application (PLA), New Drug Application (NDA), or equivalent procedure in jurisdictions outside the USA, or revision of one of the above, will likely use a selection of products from various manufacturers in their process. The end user must show the FDA that their suppliers’ processes are under control, and by inference, the products such as ultrafiltration equipment and systems, are under control.

Products, such as membrane cassettes, that are critical to an end user’s process, are subject to review by the FDA. The manufacturer of these critical products may need to supply access to their Drug Master File within their portfolio filed with the FDA.
Access

Access means that the end user submits a request to Pall Life Sciences and other suppliers of critical products, requesting that the FDA review the Drug Master File within the context of that end user’s product or process. The FDA looks into the portfolio and evaluates whether or not to accept this critical product for use in the submission.

Access does not mean that Pall Life Sciences or the FDA supplies a copy of the portfolio to the end user. Access only allows the end user to petition the FDA to evaluate the end user’s process with the Pall Life Sciences product.

Access for a submission to the FDA must be made by sending an original letter on company stationery to Pall Corporation. The letter must be signed by an officer of the end user’s company. The officer must specify which products are involved in their process. After receiving the letter, Pall Corporation issues a letter to the FDA, who performs the actual evaluation. The end user will then be advised by the FDA.

If an end user submits a letter of access to the FDA outside the routine of an NDA, PLA, IND or revision, the FDA will not review the request. Pall Corporation provides authorized access where appropriate, but customers cannot review Drug Master Files of Pall Life Sciences.

If you have questions about this process, or the results of an FDA evaluation, contact Pall.
5 Biosafety Evaluation and Test Procedures

5.1 Introduction

The purpose of the biological evaluations and tests was to evaluate the biological suitability of the materials of construction of the Omega membrane cassettes. These tests were performed by an outside contract laboratory in order to evaluate the suitability of the materials of construction of the Omega Series membrane cassette in terms of biological safety. Tests performed included the Biological Reactivity Tests, In Vivo, for Plastics (hereafter called the Biological Reactivity Tests), as described in the United States Pharmacopeia, Chapter <88>; as well as the Hemolysis Test, and the L929MEM—Cytotoxicity Test (hereafter called the cytotoxicity test). In addition, a test was also performed to measure the level of oxidizable substances and endotoxin found in a cassette after an appropriate cleaning and flushing procedure had been performed.

5.2 Summary of Test Procedures

The Biological Reactivity Tests described in the United States Pharmacopeia include injection of extracts of plastic materials, as well as implantation of the material itself into animal tissue. Four extracting media are listed which simulate parenteral solutions and body fluids. These include: (1) sodium chloride injection, (2) 1-in-20 solution of alcohol in sodium chloride injection, (3) polyethylene glycol 400, and (4) vegetable oil (sesame or cottonseed oil). Extracts are prepared at one of three standard conditions: 50 °C for 72 hours, 70 °C for 24 hours, or 121 °C for one hour. Since Omega membrane cassettes have a recommended operating temperature limit of 50 °C, cassette components were extracted at 70 °C to provide for the most stringent test condition not resulting in physical changes in the plastic itself.

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 1-in-20 solution of alcohol in sodium chloride injection extracts were injected intravenously. Vegetable oil extract and polyethylene glycol 400 extract were injected intraperitoneally. An intracutaneous test was performed to evaluate the potential of a single injection of an extract to produce tissue irritation. The four specified extracts were used.

Implantation was also performed in order to subject the materials of construction to the most stringent conditions included in the United States Pharmacopeia. Each of the components of the filter cassette was implanted separately. The hemolysis test and cytotoxicity test were conducted to determine the potential toxicity resulting from direct contact of the materials of construction with blood or tissue. The hemolysis test determines the degree of red blood cell lysis caused by contact of the test material. Using cell culture techniques, the cytotoxicity test determines the lysis of cells and the inhibition of cell growth caused by extracts of the test materials.

Endotoxin and oxidizable substance assays were performed in duplicate to show the level of these substances in filtrate from Omega (100 kDa) membrane cassettes after a recommended flushing procedure. Endotoxin in the filtrate from cassettes that have been flushed with 250 L/m² (25 L/ft²) of water per membrane area was measured using the Thermomax Chromogenic Assay Procedure test (Section 9.3: Endotoxin Assay Procedure — ThermoMax Chromogenic Assay on page 50). Oxidizable substances were measured in the same flushing solution using a colorimetric assay (potassium permanganate in a sulfuric acid acidified test solution).

5.2.1 Results

All Omega membrane cassette components were found to meet the requirements of the USP, In Vivo Class VI-70 °C Plastics. Additionally, test samples meet the requirements of the hemolysis test and cytotoxicity test. A bacterial endotoxin level of 0.018 EU/mL was eluted from the test article after cleaning and flushing. No endotoxin was measured in the filtrate. No oxidizable substances were detected in the filtrate. The tests were conducted by Toxikon Laboratories of Woburn, Massachusetts.
Results from the following tests are included in the appendices:
• USP XXI Biological Test for Plastics, In Vivo, Class VI (Section 10.1 on page 53)
• L-929-MEM Cytotoxicity Test (Section 10.2 on page 58)
• Hemolysis Test—Direct Contact with Rabbit Blood (Section 10.3 on page 61)
• Endotoxin Levels and Total Oxidizable Substances (Section 10.4 on page 64)

5.3 **Materials of Construction Conformance Summary**

- Polyethersulfone — Meets FDA 21 CFR, part 177.2440, USP Class VI plastics at 70 °C.
- Polyolefin membrane support — Meets 21 CFR, part 176.170, 177.1520, 177.2800, USP Class VI plastics at 70 °C.
- Polyester screen — Meets 21 CFR, part 177.1630, USP Class VI plastics at 70 °C.
- Polyurethane encapsulant — Meets 21 CFR, part 175.103, 175.300, 177.2600, USP Class VI plastics at 70 °C.
- Silicone gaskets—Platinum cured, medical grade, Meets 21 CFR, part 177.2600, USP Class VI plastics at 70 °C.
- Glycerin — CP/USP grade, plant origin, (added as humectant; removed with flushing).
- Sodium azide — 0.05 – 0.1%, (added as bacteriostat, removed with flushing).
6 Operational Procedures

6.1 Installing Cassettes in Holders

Follow these steps to install Omega membrane cassettes in holders. The exact procedure may vary with different holders:

1. Remove the top plate of the cassette holder and ensure the sealing surfaces are clean and undamaged.
2. Wet the silicone gaskets with deionized or pharmaceutical grade water. Place one gasket against the manifold plate of the holder and align the holes in the gasket with the holes in the manifold.
3. Place the cassette into the holder on top of the installed gasket, and place the second gasket on top of the cassette. Align the holes in the gasket with the holes in the cassette.
4. If your application requires multiple cassettes, place the next cassette on top of the gasket on the first cassette. Place another gasket on top of the cassette. Repeat this process for each additional cassette.
5. Check the alignment and orientation of the cassettes and gaskets to the holder. Place the end plate on the holder.
6. Add tie-rod spacers if required, and hand-tighten the hex nuts on the tie rods. Manually or hydraulically compress the holder closed as described in the following sections.

Pall supplies two gaskets with each cassette. Installing the first cassette requires two gaskets. Installing each additional cassette requires only one gasket. Save extra gaskets to replace worn or damaged gaskets. Pall recommends replacing gaskets after six months of use.

6.2 Cassette Holder Torque Specifications

Membrane cassettes and gaskets are installed in a holder and the holder must be compressed to exert sufficient force on the cassettes to prevent leaking when the cassette is operated under pressure. If insufficient compression is applied, air or liquid leaks from the holder. If excessive compression is applied, cassette damage may occur. The appropriate torque value depends on several factors including the operating pressure, the number of cassettes installed and the condition of gaskets and holder surfaces. Using the lowest acceptable torque value helps to prolong the useful life of a cassette.

6.3 Tightening Manual Torque Cassette Holders

Torque Value Selection and Tightening Sequences

Manual-torque cassette holders require proper torquing to prevent leaks and ensure consistent performance. Over-tightening a cassette can result in permanent damage. Therefore, you should normally use the lowest torque value that will assure system integrity. Select the recommended torque range from Table 20: Recommended Torque Values for Manual-torque Cassette Holders(15) on page 33.

Never exceed the torque limits shown—excessive torque shortens the operating life and degrades performance of membrane cassettes. Cassette holders require adjustment periodically as torque decreases over time due to cassette and gasket compression or changes in temperature. Changes tend to be greatest for new cassettes.

After you hand-tighten the assembly nuts, use a calibrated torque wrench to tighten each nut according to the specific pattern recommended for your holder (Figure 17: Pall Manual-Torque Cassette Holders and Torquing Sequence on page 33). As you tighten the nuts with the torque wrench, do not turn the nuts more than ¼ turn at a time. After reaching a ¼ turn, move and begin tightening the next nut in the specified sequence. Tighten the nuts in this fashion until the specified torque value is reached.

You must recheck the torque prior to using a cassette/holder assembly. For manual assemblies, checking torque prior to use is essential. Processes where temperature of the process fluid varies may require periodic adjustment of torque due to expansion and contraction of cassettes and gaskets.
6.4 Tightening Auto-Torque (AT) Holders

To tighten a Pall auto-torque holder, select and apply the required hydraulic pressure to the hydraulic cylinders on the holder (Table 21: Recommended Hydraulic Pressure Range for Pall AT Cassette Holders). Refer to the AT-holder operating instruction manual for the detailed operating procedure. If you are using the Pall Hydraulic Pump, refer to the owner’s manual for operating procedures. Auto-torque assemblies do not need periodic pressure adjustment. The AT system eliminates possible operator error that can occur with a manual torque cassette holder. The AT system maintains the specified clamping force under conditions that would cause a manually-torqued cassette to require re-torquing (i.e., temperature reduction or cassette-gasket compression).
### Table 21: Recommended Hydraulic Pressure Range for Pall AT Cassette Holders

<table>
<thead>
<tr>
<th>Holder Type</th>
<th>No. Hydraulic Pistons on Holder</th>
<th>Recommended Hydraulic Pressure Range for AT Holders (psi)</th>
<th>(bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Centrasette™ LV AT</strong></td>
<td>4</td>
<td>500 – 800</td>
<td>34 – 54</td>
</tr>
<tr>
<td><strong>Centrasette™ 5 AT</strong></td>
<td>4</td>
<td>500 – 800</td>
<td>34 – 54</td>
</tr>
<tr>
<td><strong>Centrasette™ 10 AT</strong></td>
<td>2</td>
<td>1100 – 1600</td>
<td>75 – 110</td>
</tr>
<tr>
<td><strong>Centrostak™ AT</strong></td>
<td>2/level</td>
<td>1100 – 1600</td>
<td>75 – 110</td>
</tr>
<tr>
<td><strong>Maxisette™ AT</strong></td>
<td>6</td>
<td>1400 – 1800</td>
<td>95 – 122</td>
</tr>
</tbody>
</table>

(17) Recommended hydraulic pressure ranges for these holders with Omega cassettes have been lowered from previous versions of this guide. Changes are based on studies indicating reduced pressures will sufficiently compress cassettes in the holder.

(18) For long-term storage of cassettes in the AT holder, reduce the hydraulic pressure value by 20 to 30% of the recommended value.

### 6.4.1 Effect of Temperature on Set Torque Value

At a given torque setting, changes in temperature will change the clamping force exerted by the holder on the gaskets and cassettes. If the temperature decreases more than 5 °C from the temperature at which cassette was last torqued, re-torque the holder. If the temperature will increase more than 20 °C, use lowest possible starting torque that maintains system integrity.

### 6.5 System Hold-up Volume

It is imperative to determine the hold-up volume and minimum working volume for your system following installation of cassettes and prior to flushing out the system.

**Feed/Retentate Hold-up Volume** is the total volume, most of which is recoverable, contained within the feed/retentate flow path. The feed/retentate flow path includes the cassette feed/retentate channels, holder ports, pump, valves, gauges, connectors, and tubing. You must determine the system hold-up volume of your system because each installed system varies due to specific applications (Figure 18: TFF System Schematic with Feed/Retentate Flow Path on page 35).

**Minimum Working Volume** is the hold-up volume plus a minimum volume of liquid that must remain in the bottom of the feed tank at the operating flow rate to prevent air from being drawn into the cassette system.

The minimum working volume limits the maximum concentration factor achievable. It is affected by the crossflow rate. At a higher crossflow rate, a greater liquid volume in the bottom of the feed tank is required to prevent air from getting drawn into the pump. Tank design significantly affects the minimum volume required to prevent air from getting into the system.

**Permeate Hold-up Volume** is the total volume contained within the permeate flow path.

**Non-Recoverable Volume** is the volume remaining in the Feed/Retentate flow path after the flow channel has been pumped out and drained. Optimization of the product recovery step will ensure high product recovery.

Procedures for determining Feed/Retentate Hold-up Volume, Permeate Hold-up Volume, and System Minimum Working Volume are detailed in Section 3.3: Hold-up Volume and Minimum Working Volume of the Membrane Cassettes Care and Use Procedures (R00640 Rev B).

### 6.6 Recommended Crossflow Rates for Pall Membrane Cassettes

Table 22 lists recommended crossflow flux (CFF) rates for operating Pall TFF membrane cassettes. Other parameters such as TMP and temperature can be evaluated at these CFF values.

\[
\text{CFF} = \frac{\text{L/min [retentate flow rate]}}{\text{ft}^2 \text{ or m}^2 [\text{membrane area}]} 
\]

The values listed under Processing Mode are recommended for use when processing a sample. Higher flow rates are recommended for cleaning and sanitization (Cleaning Mode).
Operational Procedures

6.7 Sanitization

Cassettes are not supplied sterile nor does Pall claim they are pyrogen (endotoxin) free. New cassettes contain 0.05 – 0.1% sodium azide as a bacteriostatic agent to prevent bacterial growth. Cassettes should be washed free of storage agents, sanitized, and washed free of any trace of pyrogens prior to use. After use, cassettes are normally stored in an aqueous solution of 0.1 N sodium hydroxide to prevent bacterial growth.

Method for Sanitization

The recommended procedure for sanitizing and depyrogenating cassettes is described in Membrane Cassette Care and Use Procedures.

6.8 Flushing

Flushing removes storage agents or cleaning agents from the cassette prior to use in order to prevent contamination of the product.

Flushing Method

The recommended method for flushing is described in Membrane Cassette Care and Use Procedures.

6.9 Integrity Testing

Integrity testing enables you to determine that the filtration system components and membranes are not leaking or damaged. You can perform two types of integrity test:

**Table 22: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes**

<table>
<thead>
<tr>
<th>Holder Type</th>
<th>Units</th>
<th>Minimum CFF Processing Mode</th>
<th>Recommended CFF(19) Processing Mode</th>
<th>Recommended CFF(19) Cleaning Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L/min/m²</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
</tr>
<tr>
<td>Centramate™ or Centrasette™</td>
<td>3</td>
<td>8</td>
<td>5 – 8</td>
<td>10 – 20</td>
</tr>
<tr>
<td></td>
<td>L/min/ft²</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.8</td>
<td>0.5 – 0.8</td>
<td>1.0 – 2.0</td>
</tr>
<tr>
<td>Maximate™ or Maxisette™</td>
<td>2</td>
<td>5</td>
<td>4 – 5</td>
<td>7 – 15</td>
</tr>
<tr>
<td></td>
<td>L/min/ft²</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
<td>Screen Channel Cassettes</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.5</td>
<td>0.4 – 0.5</td>
<td>0.7 – 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Suspended Screen Channel Cassettes</td>
<td>Suspended Screen Channel Cassettes</td>
<td>Suspended Screen Channel Cassettes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6 – 1.0</td>
<td>1.0 – 2.5</td>
<td></td>
</tr>
</tbody>
</table>

(19) Trials must be performed to determine the most effective crossflow rate to use for any specific application.
1. A system integrity test indicates the presence of air leaks in the system, such as in the holder, fittings, and gaskets.
2. When performed after a system integrity test, the membrane integrity test indicates the absence of holes, tears, seal failures, or defects in the membrane cassette.

The membrane integrity value is a measurement of air that either diffuses through the liquid that fills the pores in the membrane or passes through pinholes or defects in the membrane or around the seals. Membrane integrity measurements are made at a specified air (or nitrogen) pressure. The diffusive membrane integrity value is related to pore size, pore density, membrane density (surface skin thickness), temperature, and pressure. However, differences in membrane integrity values due to these factors are small compared to the large increases that result from the presence of even a tiny pinhole.

Prior to performing the membrane integrity test, you must flush and condition the tangential flow filtration system with the cassettes installed. The method and equipment you use can also affect results. Pall recommends using mass flow meters connected to the upstream side of the cassette to yield accurate and consistent results.

The schematic in Figure 19: Membrane Integrity Test Setup Using a Pall Integrity Analyzer on page 36 shows an integrity test setup using the Pall Integrity Analyzer. This instrument regulates the air flow rates and pressures to perform the integrity tests. A sensitive mass flow meter measures and displays the rate of air that is diffusing through the membrane. The following sections describe how to perform the system integrity test and the membrane integrity test.

### 6.9.1 System Integrity Test

During the integrity test, you pressurize the system with compressed air, and monitor the airflow. The airflow should gradually drop to zero. Test the integrity of the system before testing the integrity of the membrane. There should be little to no air flow if the system is sealed.

**Integrity Test Method**

The system integrity test procedure is described in *Membrane Cassette Care and Use Procedures*.

### 6.9.2 Membrane Integrity Test

The membrane integrity test checks the membrane and internal seal assemblies of the cassette. The cassette must be fully wetted-out prior to making a measurement. The pressure on the feed/retentate side of the membrane should be set to the appropriate value prior to opening the filtrate valve. The membrane integrity measurement should be made within fifteen minutes of having the water expelled from the feed/retentate channel to prevent the pores from drying out. You should perform the system integrity test before performing a membrane integrity test.

The membrane integrity test should be performed separately on each cassette. When multiple cassettes are used in one system, the cassettes can then be installed and both a system and membrane integrity test performed. The air
Forward Flow values obtained for the combined stacks of cassettes should be approximately equal to the sum of the individual air Forward Flow values. If after use, the air Forward Flow values obtained for the stacks increases by more than the limit value for one cassette, the stacks should be broken down and each cassette tested separately. This is done to eliminate the possibility that there is a single defect in one cassette that accounts for the total increase in Forward Flow.

**Method**

The membrane integrity test procedure is described in *Membrane Cassette Care and Use Procedures*.

### 6.10 Method for Determining Water Flux and Normalized Water Permeability

Membrane water flux is a measure of the permeability of the membrane to clean water at a specified transmembrane pressure and temperature.

**Method**

Procedure to determine water flux and normalized water permeability is described in *Membrane Cassette Care and Use Procedures*.

### 6.11 Cleaning the System and Cassettes

Proper cleaning of cassettes after use is critical to their reuse. You must establish a cleaning protocol that removes foulants and recovers the initial water permeability. The key cleaning parameters include:

- Cross flow rate
- Temperature
- Cleaning agent and concentration
- Time

#### 6.11.1 Cross Flow (Retentate Flow Rate)

Increasing cross flow rates improves cleaning effectiveness. Pall recommends a cleaning cross flow rate of 1.5 – 2 times the normal operating cross flow rate. The retentate valve should be fully open. For suspended screen cassettes, the filtrate valve should not be completely closed. Since most foulant is lodged at the surface of a skinned, anisotropic membrane, the objective is not to drive it into the membrane but to hydrolyze, oxidize, or resolubilize the material and remove it.

#### 6.11.2 Cleaning Agent and Concentration

The choice of cleaning agent depends on the possible foulant. Sodium hydroxide at concentrations up to 0.5 N usually is sufficient to remove most biological foulant. The addition of 400 ppm of sodium hypochlorite may help remove additional materials not removed with NaOH alone. This mixture is particularly effective for removing cells and cellular debris from the cassette. A list of recommended cleaning agents and the type of foulants they may remove is presented in *Membrane Cassette Care and Use Procedures*. In general, higher concentrations will improve cleaning and/or reduce time. However, cassette life may be compromised, especially when using concentrated solutions at elevated temperatures.

#### 6.11.3 Temperature

Increasing temperature normally improves cleaning significantly. If possible, heating and maintaining the temperature of cleaning solutions between 35 – 45 °C is strongly recommended. If the foulant is very hydrophobic, reducing the temperature may sometimes help.

#### 6.11.4 Time

Cleaning should take 30 – 120 minutes. If a membrane recovery of greater than 80% of initial water permeability cannot be established within two hours, it may indicate that a different cleaning agent is required. The time required to achieve the required level of cleaning should be established as part of a carefully performed cleaning study.
6.12 Recommended Cleaning Procedures

Procedures for flushing and cleaning cassettes after use are described in the Membrane Cassette Care and Use Procedures.

6.13 Cleaning Study Protocol

You should perform a cleaning study to identify the cleaning time, temperature, and concentration that provide the optimal recovery of the initial water permeability. The following steps describe, in general, how to perform a cleaning study. Actual times and volumes can be changed:

1. After recovering concentrated product, open retentate valve, and close filtrate valve (screen channel cassettes only). Flush the cassette to waste with 10 – 20 L/m² of spent filtrate; or if product was in filtrate, with fresh buffer.
2. Add additional spent filtrate or buffer to the feed reservoir (5–10 L/m²). Circulate it for 15 minutes at twice the cross flow rate used for processing. Spent filtrate makes a good flushing agent because it was used to solubilize the product initially and saves the cost and time of making up fresh buffer. Furthermore, the spent filtrate was already ultrafiltered and may be cleaner than fresh buffer. Direct the retentate and permeate to drain. Pump the system dry.
3. Select a cleaning agent, concentration, and temperature. The recommended starting solution is 0.5 N NaOH, at 35 °C. Add 20 – 30 L/m² of the cleaning solution to the feed reservoir. Open retentate and filtrate valves, and flush approximately 5 L/m² of the cleaning solution to waste.
4. Set the system for recirculation and recirculate for 30 minutes. At 30 minutes, pump out the cleaning solution to waste.
5. Add 30 – 40 L/m² of water to the feed reservoir. Flush the cassette with water (10 L/m² through retentate, then 10 L/m² through filtrate). Recirculate through both retentate and filtrate for 10 minutes. Increase cross flow to increase feed pressure by about 0.2 – 0.5 barg (3 – 5 psig) to remove any air trapped in the cassette. Reduce the flow after a few seconds. Repeat several times until no air is observed exiting from the retentate or permeate lines.
6. Measure the water permeability.

If you have recovered more than 90% of the membrane’s initial water permeability, you can accept the process as is or you may try a lower concentration or temperature after the next process is run on the membrane. Less stringent conditions may prolong membrane life.

If after the cleaning, you recover less than 90% of the membrane’s initial water permeability, repeat steps 3 through 6. If the second cleaning improves membrane recovery, repeat steps 3 through 6 a third time. If the second cleaning does not improve recovery to at least 80%, you should consider changing cleaning solution; for example, adding 400 ppm NaOCl to the NaOH, and repeating the process. The inability to recover at least 80% of the initial water permeability does not necessarily mean that the cassette has not been cleaned. Cassette compression can cause a reduction in the calculated value for water permeability. It is important not to over-torque cassettes. Increasing the temperature of the cassettes after they have been properly torqued, will cause an increase in compression.
7 System Characteristics

This section describes tangential flow filtration system hardware and design considerations as well as cassette and system hold-up volumes.

7.1 System Hardware and Design Considerations

A tangential flow filtration system consists of the membrane cassette, cassette holder, pump, valves, tubing, pressure gauges, connecting piping, and reservoirs, etc. (Figure 20: Typical Tangential Flow Filtration System on page 39). If these components are improperly designed, sized, or surface-finished, cassette performance can be compromised. Contact Pall Life Sciences or your local Pall distributor for help assessing the applicability of existing systems or in designing a complete system.

7.1.1 Holder

Membrane cassettes require installation in a properly designed holder that allows fluid distribution into and out of the flow ports. The holder must also maintain separation between the feed/retentate and filtrate flow paths and must not restrict fluid flow. Port diameters and flow paths should be of sufficient diameter to prevent significant pressure drops at the maximum flow rates required to operate the maximum number of installed cassettes. Pall membrane cassette holders are designed to provide optimal performance from Pall membrane cassettes. You can use Pall cassettes in holders made by other manufacturers as long as the holder meets the flow and design requirements above. Consult the holder manufacturer’s instructions for additional information.

7.1.2 Pressure Gauges (Transducers)

Pressure gauges and transducers allow accurate measurement and recording of operating pressures throughout the tangential flow filtration process, and are needed because process pressures should be monitored and controlled from run to run. Furthermore, changes in pressure can indicate a problem, which if allowed to go unresolved, can compromise the quality of the final product. Pressure gauges should be installed on the feed, retentate and filtrate lines to accurately determine transmembrane pressure and to identify possible pressure drops. Choose and install pressure gauges so as not to cause pressure drops or add dead legs to the system. Pressure gauge scale and range should enable accurate reading. Gauges should be capable of being calibrated and should be accuracy certified. For biopharmaceutical applications, use stainless steel, glycerin filled, electropolished, diaphragm gauges with sanitary connectors. Use unfilled gauges if steam sterilization is required.
7.1.3 Valves
Valves, if improperly sized, can cause flow restrictions and hydraulic pressures that affect the transmembrane pressure and process results. They may also cause cavitation — a high flow through a narrow opening causing a pressure drop that can result in degassing of the liquid. Cavitation can result in air entering the cassettes, which reduces the effective membrane area. It can also cause foaming and product denaturation.

7.1.4 Fittings Package for Cassette Holders
A fittings package consists of pressure gauges, valves, connectors, gaskets and a common manifold for the filtrate ports (Figure 21: 3-gauge, 2-valve, Sanitary-fitting Package for a Centrasette C-5 holder on page 40). It is important that these components be properly sized to prevent pressure drops and dead legs. Complete fittings packages are available to complement each cassette holder.

7.1.5 Feed Reservoir
Feed reservoir design affects the minimum system working volume and consequently the maximum achievable concentration factor. Design considerations include:

- Vortexing — A vortex can cause air to be drawn into the feed pump, resulting in foaming, possible product denaturation and product loss. A vortex can also affect the volume to which a product can be concentrated. Addition of vortex breakers (fins) and proper positioning of the retentate line can prevent this problem.
- Mixing — Inadequate mixing may result in a density gradient forming in the feed reservoir. For example, without adequate mixing, the concentrated retentate returned to the feed reservoir can settle to the bottom, resulting in a high product concentration at the bottom. When the highly concentrated product enters the cassette, the pressure differential across the cassette increases and may affect the filtrate flux rate. Proper design and positioning of the retentate line in the reservoir or addition of a mixer can prevent this problem.
- Sedimentation — Sedimentation of precipitates or cellular debris can be pulled into cassettes on start-up causing the feed ports to plug. Proper reservoir design and positioning of the outlet and drain ports and/or addition of a suitable prefilter, if appropriate, can eliminate this problem.
- Temperature Control — Jacketed reservoirs can help control product temperature. However, depending on the design, when the product is concentrated and the reservoir liquid level is low, the jacket may not be able to maintain the required temperature. Also, condensation above the liquid level in the reservoir may form ice that could fall into the process fluid and cause foaming. While a heat exchanger may adequately control process fluid temperature, localized heating at the pump may still cause heat denaturation.
• Positioning — The location of a feed reservoir relative to the distance and height of a pump affects the operation of a system. Long feed lines cause pressure drops. Elevated feed reservoirs on upper floors may provide higher than anticipated feed pressures.

• Pipe Size — Improperly sized ports and connecting pipes can cause pressure drops. Insufficient positive pressure entering a pump can cause cavitation resulting in high shear in the process stream.

7.1.6 Feed Pump

The pump must deliver the required volumetric flow at operating feed pressures and pump speeds. Pressure and temperature, nature of the sample (clean solution versus suspended solids), solvent resistance, and material of composition contacting the product influence pump selection. Some design considerations include these:

• Flow Rate Requirement — The pump must meet both process and clean-in-place flow rate requirements. The clean-in-place volumetric flow rate may be double the process volumetric flow rate. Improperly sized pumps may not deliver required flow rates or may not operate properly at high or low flow rates due to insufficient torque.

• Shear and Heating — Pumps produce shear and impart heat to the process solution. Hence, running a pump at low speeds reduces heating and shear. The pump must be properly sized for this application (i.e., matching the flow requirement to pump speed, pressure, etc.). Impeller design influences performance. In rotary lobe pumps, a tri-lobe versus bi-wing may give different results with regard to slippage, pulsation, heating, and shear. This may be significant when working with heat-sensitive molecules or living cells.

7.1.7 Piping

The following describe some piping considerations:

• Pipe Diameter — The internal diameter of piping must be chosen to minimize frictional effects and not add a significant pressure drop to the system. At the same time it is important to maintain a minimum hold-up volume to minimize post-wash dilution and maximize volume reductions. Systems are sized so that the linear flow rate through the piping is approximately 2–5 m/s for process conditions and 5–8 m/s for cleaning. If the system is designed to hold varied amounts of membrane, the piping should be sized for the maximum load. This will result in a larger than desired hold-up volume when working with minimum membrane area. It is possible to design the system so that the piping and valves can be changed for different configurations.

• Pipe Length — Pressure drop is directly proportional to length of piping. Doubling the length doubles the pressure drop related to the piping. If long runs of piping are used, it is important to consider how it will affect the process.

• Finish — For pharmaceutical applications, electropolishing of internal surfaces to a finish of 25 μinch RA (0.6 μm RA) or better is normally specified to minimize metal leaching, friction and to improve cleaning. This applies to the piping and reservoirs.

• Bends and Turns — Each bend adds to the pressure drop in a line. Utilize minimal bends and turns.

7.1.8 Heat Exchanger

Heat exchangers are used on systems to adjust and maintain the temperature of the process fluid. The temperature of process fluids may be raised or lowered depending on process requirements. Fluid temperature is usually lowered to maintain the biological activity of products. The position of heat exchangers can be critical. Heat can be introduced from the external environment and more importantly, from the pump. Addition of a cooling jacket to the feed reservoir or a heat exchanger coil on the retentate line is common. Since a high heat input may come from the pump, even though the temperature of the feed solution is properly maintained, the product can be denatured at the pump. Therefore, properly size the pump to reduce localized heating. It may also be practical to cool the solution as it is fed into the pump to compensate for the heat input.

7.2 Cassette and System Hold-Up Volume

The cassette and system hold-up volumes affect the maximum concentration factor that can be achieved for a systematic a given sample volume, the smaller the system hold-up volumes greater the concentration factor that you can obtain. Reducing hold-up volumes can translate to improved product yield.
7.2.1 Cassette Hold-up Volume

The membrane cassette hold-up volume represents the total volume within a cassette’s flow channels and input and output ports. The feed-retentate hold-up volume is the volume contained in the feed-retentate flow channels and the feed input and output ports. The filtrate hold-up volume is the volume contained in the filtrate flow channels and output ports (Table 23: Omega Cassette Hold-up Volumes).

Table 23: Omega Cassette Hold-up Volumes

<table>
<thead>
<tr>
<th>Channel</th>
<th>Screen Type</th>
<th>Centramate</th>
<th>Centrasette</th>
<th>Maximate</th>
<th>Maxisette</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.09 m²</td>
<td>0.46 m²</td>
<td>1.9 m²</td>
<td>2.4 m²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1 ft²)</td>
<td>(5 ft²)</td>
<td>(20 ft²)</td>
<td>(25 ft²)</td>
</tr>
<tr>
<td>Feed-Retentate</td>
<td>Fine</td>
<td>18</td>
<td>90</td>
<td>360</td>
<td>450</td>
</tr>
<tr>
<td>Feed-Retentate</td>
<td>Medium</td>
<td>18</td>
<td>90</td>
<td>360</td>
<td>450</td>
</tr>
<tr>
<td>Feed-Retentate</td>
<td>Suspended</td>
<td>35</td>
<td>175</td>
<td>700</td>
<td>875</td>
</tr>
<tr>
<td>Filtrate</td>
<td></td>
<td>18</td>
<td>90</td>
<td>360</td>
<td>450</td>
</tr>
</tbody>
</table>

7.2.2 System Hold-up Volume

The system hold-up volume is the total volume of liquid in the feed/retentate flow path of the system. It is equal to the combined volumes in the cassette feed/retentate channels, holder ports, pumps, valves, gauges, connectors, and tubing which make up the feed/retentate flow path. Product contained within this flow path can be recovered by displacement with an appropriate buffer or by an air purge.

System users must determine the system hold-up volume due to the uniqueness of each installation. The method for determining system hold-up volume is described in Membrane Cassette Care and Use Procedures.
8 Method Details

8.1 Procedure for Determination of Extractables from Omega Membrane Cassettes

8.1.1 Equipment

- Maximite holder and gauges
- Peristaltic or diaphragm pump with inert contact surfaces
- Inert (PTFE or FEP) tubing and adapters to sanitary flange connections
- Silicone tubing
- Borosilicate glassware
- Timer/stopwatch
- TOC meter (using CO₂-specific detection)
- pH meter
- Rotary flash evaporator
- Vacuum source with trap
- Muffle furnace
- Drying oven
- Desiccator
- Ceramic crucibles with covers
- Analytical balance
- UV-Visible spectrophotometer
- Infrared spectrophotometer
- Particle-free sample bottles (250 or 500 mL)

8.1.2 Reagents and Materials

- Omega 300 kDa membrane Maximite screen channel cassettes (OS300G01)
- Silicone gaskets — Maximite
- Pall 0.2 μm Kleenpak™ filter capsules (P/N KA2NFP1)
- Sodium hydroxide (reagent grade)
- Sodium hypochlorite solution (5.25%)
- DI water source

8.1.3 Preparation of Apparatus and Materials

1. Thoroughly clean glassware and the liquid contact surfaces of pumps, tubing, and holder. Where appropriate, a TOC measurement of a DI water rinse is used to assure cleanliness. Clean the porcelain crucibles by heating in furnace at 500 °C or higher for approximately 30 minutes and allow to cool in a desiccator.

2. Prepare sanitizing solution of 0.1 N NaOH/200 ppm hypochlorite by dissolving 16.8 grams of NaOH in 3 liters of DI water, adding 16 mL of 5.25% sodium hypochlorite (commercial bleach), and bringing the total volume to 4 liters. Filter solution through a 0.2 μm filter.

3. Prepare 25% ethanol extraction solution by adding 1.1 liter of 95% ethanol USP to 2.9 liters filtered DI water.

8.1.4 Extraction Procedure

1. Lubricate a test item (either a cassette, or for the negative control, a set of cut-out gaskets only) with DI water and install the test item in the cassette holder. Connect the holder to a filtered DI water source. Adjust the flow to achieve 2 L/min. Adjust the retentate valve to obtain 90% filtrate/10% retentate and flush 25 liters to a drain.

2. Place pump inlet, filtrate line, and retentate line into a vessel charged with 4 liters of sanitizing solution at 20 – 25 °C and recirculate at 1 L/min. Open the filtrate valve. Adjust the retentate valve to obtain 0.7 barg (10 psig) on the retentate pressure gauge and recirculate for 30 minutes. Stop the pump, open retentate valve, and drain the system.
3. Connect the inlet to a filtered DI water source. Adjust the flow to achieve 2 L/min, adjust the retentate valve to obtain 90% filtrate/10% retentate, and flush to a drain at 2 L/min for 10 minutes. Take a water sample at the permeate port and measure TOC and pH. If the TOC is over 1 ppm or if the pH is above 8, continue flushing for an additional 10 minutes and check effluent quality again. Repeat the flush until the TOC and pH fall below these limits. Drain the system.

4. Fill a reservoir with 2 liters of extraction solvent (either DI water or 25% ethanol) and bring to the test temperature. Place the pump inlet tubing, filtrate line and retentate line into the reservoir. Using inert tubing, pump the extraction solution through the system at 2 L/min, adjusting the filtrate valve to obtain 10% of total flow through the filtrate line.

5. Continue recirculation for 16 hours. At the end of this time, pump the extraction solvent into a clean vessel labeled with the solvent name, cassette serial number, and "extract 1," and place 2 liters fresh solvent into the reservoir. Allow the solvent to reach the test temperature, then recirculate the fresh solvent for 16 hours. Pump the second extract into a clean vessel labeled with the solvent name, cassette serial number, and "extract 2."

6. This "rinse, sanitization, and extraction" procedure is repeated for 2 cassettes and one negative control (gaskets only) using water as an extracting solvent, and for 2 cassettes and one negative control (gaskets only) using 25% ethanol as extracting solvent. In addition, a positive control consisting of between 45 and 55 mg of potassium acid phthalate dissolved in 2 liters of each solvent was prepared to show the quantitative recovery of a nonvolatile organic compound.

### 8.2 Procedure for Determination of Nonvolatile Residue

1. Take an aliquot of the extraction solution (e.g., 1000 mL) and evaporate on a rotary evaporator using a clean 1000-mL, glass, round-bottom flask. Adjust and maintain the temperature of the water bath to 80 °C. Evaporate the aliquot to less than 25 mL.

2. Weigh crucibles to the nearest 0.0002 g. Repeat until constant weight is obtained (± 0.0002 g). Store in a desiccator.

3. Quantitatively transfer the concentrated extract to the tared crucible contained in the desiccator. If residue remains in the round-bottom flask, add a few drops of fresh DI water, swirl, and add to crucible. If more than a few drops are needed, note the volume used.

4. Carefully place the crucibles in an oven (circulating air type) maintained at 60 – 80 °C. Evaporate the extract to dryness. Remove the crucibles from the oven after evaporation of the water, return to a desiccator, cover, and allow to cool to room temperature.

5. Weigh the crucibles to the nearest 0.0001 g and record the weight.

6. Calculate the nonvolatile residue (NVR) for the volume evaporated as follows:

\[
NVR_V (\text{mg}) = CR (\text{mg}) - CC (\text{mg})
\]

Where \(NVR_V\) = NVR for volume evaporated in mg

\(CR\) = constant weight of crucible and residue

\(CC\) = constant weight of crucible

7. Calculate the total NVR for both the control and each sample.

\[
NVR_T (\text{mg}) = NVR_V (\text{mg}) \times V_I/V_E
\]

Where \(NVR_T\) (mg) = total NVR in mg

\(V_I\) = initial solvent volume used for extraction

\(V_E\) = volume of solvent taken from final volume for evaporation

8. Calculate the net NVR for each sample as follows.

\[
\text{Net NVR (mg)} = NVR_S - NVR_C
\]

Where \(NVR_S\) = total nonvolatile residue of sample in mg

\(NVR_C\) = total nonvolatile residue of control in mg

### 8.3 Effectiveness of the Flushing Protocol for Removing Storage Agents

The following steps show how to determine the effectiveness of the flushing and sanitization protocol that is used to remove storage agents from Maximate cassettes:
1. Install and torque the Maximate cassette into a Maximate holder per the Maximate Membrane Cassettes Operating Instructions. Torque specifications should be 5.65 Nm (50 in-lb) for the 8-bolt holder and 11.3 Nm (100 in-lb) for the 4-bolt holder. Direct the retentate and filtrate streams back to the feed (Figure 22).

2. Flood the feed, retentate, and filtrate streams. Fill feed reservoir with 2 L of 0.1 N NaOH. Fully open the filtrate and retentate valves. Start pumping and increase the feed flow rate until solution flows through both the retentate and filtrate streams.

3. Apply 0.7 barg (10 psig) retentate pressure by closing the retentate valve. Run until air bubbles are no longer visible in the filtrate stream. Open retentate valve completely, then close the filtrate valve. Increase the retentate flow rate to 2 L/min. Run for approximately one minute to allow the air bubbles to be purged. Reduce flow rate to 1 L/min.

4. Open the filtrate valve completely and then close retentate valve until the retentate flow rate is approximately 0.1 L/min. Adjust pump flow to maintain a feed pressure of 2.0 barg (30 psig). Run for 30 – 45 minutes.

5. Flush the cassette and assembly with DI water to waste. Replace the feed reservoir with a vessel containing 12 – 15 L of DI water. Direct the filtrate and retentate streams to waste (Figure 25).

6. Open the retentate valve completely. Close the filtrate valve. Adjust the pump to give a retentate flow of 2 L/min. Flush at least 10 L to waste while taking a 30 mL flush sample for analysis after every 0.5 liters. Stop pump after taking the last sample. Do not allow the test system to run dry.

7. Flush the filtrate stream to waste—Refill feed reservoir with 12 – 15 L of DI water. Adjust feed pump flow to approximately 1 L/min. Open filtrate valve and then close retentate valve until the retentate flow rate is approximately 10 mL/min. Adjust pump speed to maintain a feed pressure of 2.0 barg (30 psig). Flush at least 10 liters to waste through the filtrate line while taking a 30 mL flush sample for analysis after every 0.5 liters.

8. Determine the glycerin concentration of the retentate and filtrate samples by high performance liquid chromatography. Determine the sodium azide concentration by the spectrophotometric method.

8.4 Determining Solute Passage on Membrane Disk

This section describes the procedure for determining solute passage on disc membranes.

8.4.1 Introduction

Solute passage is the key parameter related to the proper functioning of a membrane. It is related to the pore size distribution in the membrane. Solute passage is affected by solute concentration, temperature, pH, ionic strength and pressure.

Therefore, it is important to control these parameters when measuring solute passage.
8.4.2 Materials and Equipment

- Millipore 8050 stirred cells
- 50 mL graduated cylinders
- Stopwatches, capable of measuring 0.001 minutes
- UV/VIS spectrophotometer
- Refractometer
- Calculator
- Regulated air supply
- Stir plates

8.4.3 Chemicals

Required solutes depend on the membrane to be tested. Consult the Membrane Solute Passage Specifications section (Table 8 on page 9) and Test Solute Concentration and Detection Method (Table 9 on page 9). Ultrapure water is required. Water quality should be at a minimum distilled, DI, and ultrafiltered through a 10 K membrane.

8.4.4 Preparation of Test Solutions

Various solutes are used to test passage and retention characteristics. The choice depends on the molecular weight cut-off or pore size of the membrane. The solutes and concentrations used for testing Omega membranes are provided in Section 2.9: Test Solute Concentrations and Detection Methods on page 9.

Protein Solutions

Prepare BSA test solution in phosphate-buffered saline. Prepare IgG in saline (0.85%) as the solvent. Protein solutions may be placed in the refrigerator and allowed to dissolve without the aid of stirring or on a stir plate with very slow speed to avoid denaturing the protein. You can store protein solutions at 4 – 8 °C for up to a week.

Non-Protein Solutions

Polyvinylpyrrolidones (PVPs) and blue dextran are made with DI water as the solvent. PVPs dissolve rather rapidly in water and may be stirred. In solution they can be stored at room temperature for 10 days. Blue dextran dissolves slowly and needs constant stirring. It should be refrigerated and can be stored for up to 10 days. Latex beads are made in DI water with 0.020 – 0.025 g of Triton X-100 per 200 mL. The beads may be shaken or stirred and can be stored at room temperature for 10 days.

8.4.5 Procedure

Follow these steps to determine solute passage:

1. Set up the process by punching out a 43 mm disc from a membrane test sample. Assemble the stirred cell with the membrane facing skin side (shiny side) up. Fill the stirred cell with DI water (24 – 26 °C) and attach the top.
2. Prepare for the water flux by setting the pressure at 3.7 barg (55 psig) for molecular weights from 1 to 100 kDa, 10 psig for molecular weights from 100 to 1000 kDa, and 0.24 barg (5 psig) for molecular weights from 1000 kDa to 0.16 μm. Flush 40 – 50 mL of DI water through the disc to remove storage agents. Open unit, add 50 mL of water (24 – 26 °C) and reseal. Adjust the speed of the stir plate until a vortex reaches down approximately one third of the volume from the top.
3. Pressurize the cell to the appropriate pressure. Allow approximately six drops of water to pass into a waste container. On the seventh drop insert the graduated cylinder under the outlet tube and start the timer. When 45 mL of filtrate has been collected, stop the timer and remove the outlet tubing from the cylinder. Record the elapsed time and the collected volume. Calculate the water flux in mL/min/cm². (On a Millipore Model 8050 the area of the exposed membrane is 13 cm²).
4. After performing the water flux, the cells are emptied and the outlet hose should be shaken to rid it completely of water. Pour 30 mL of solute into each cell. Adjust stirring to create a vortex not to exceed 1/3 the volume of liquid in the cell. Pressurize the cell to the correct pressure.
5. Let approximately six drops of solute pass into a waste container to rid the outlet tube of residual water and air. On the seventh drop, start the timer and insert the graduated cylinder under the outlet tube of the stirred cell. When the permeate volume equals 15 mL, stop the timer and remove the tubing from the cylinder. Record the time and the exact volume collected. Take sample of permeate.
6. Calculate the solute flux in mL/min/cm².

8.4.6 Analysis and Interpretation of Data

The percent of solute passage is determined through the use of a UV/Visible spectrophotometer when testing with proteins, blue dextran, or latex beads; a differential refractometer is used when testing with PVPs or Bacitracin. Both instruments are calibrated with the solvent as the zero or base, and the test solution as the 100% concentration. Record the absorbance or refractive index of the standard test solution. Measure the absorbance or refractive index of the collected samples.

Determine the percentage of solute passage using the following formula:

\[
\text{Passage} = \left( \frac{\text{absorbance or RI collected sample}}{\text{Absorbance or RI Std. test solution}} \right) \times 100
\]

Where RI = refractive index

8.5 Determining Solute Passage on Membrane Cassettes

8.5.1 Introduction

You can measure the solute passage in a cassette. The values you obtain will be different from those obtained on disc due to differences in operating conditions including transmembrane pressure and cross flow. Changing the transmembrane pressure or crossflow can alter the solute passage significantly. However, if operating conditions are established, and solute passage is measured on a new cassette using set parameters, an initial value can be established. You can then repeat the solute passage measurement on a used cassette, compare the results to the initial value, and determine if the cassette’s performance has changed.

8.5.2 Materials and Equipment

- Membrane cassette and silicone gaskets
- Cassette holder and tangential flow filtration system
- UV–Visible spectrophotometer or refractometer
- Sample tubes
- Graduated cylinders
- High purity water

8.5.3 Preparation of Test Solutions

Prepare test solutions according to the specifications provided on Table 8: Solute Passage Specifications and Table 9: Concentration of Solute and Detection Method Used to Test Membranes on page 9.

8.5.4 Procedure

Follow these steps to determine the solute passage.

Cassette Preparation

Prepare the cassette and hardware as described in Preconditioning Cassettes and System on page 17 of the Membrane Cassette Care and Use Procedures. Include sanitization, flushing, normalized water permeability, and buffer conditioning using the appropriate solutions for the test solute. If the cassette has been used, perform the cleaning protocol first followed by thorough flushing with water.

Passage test

1. Drain the system and set it up for recirculation.
2. Open the retentate and filtrate valves completely.
3. Add the test solution to feed reservoir. Use approximately 4 L/m², but not less than twice the system hold-up volume. Close the filtrate valve. Adjust pump speed and retentate valve to establish a feed pressure of 2 barg (30 psig) and a retentate pressure of 0.7 barg (10 psig).
4. Allow the solution to circulate for five minutes.
5. Take a sample of the retentate (initial recirculation solution). Very slowly (over 15 – 30 seconds) open the filtrate valve. Carefully increase the pump speed to reestablish the 2 barg/0.7 barg (30/10 psig) pressures. (Monitor the filtrate pressure. When you first start to open the valve, nothing will happen. When the pressure starts to drop, continue to open the filtrate valve very slow and carefully.)
6. After the filtrate valve is fully open, let the solution circulate for five minutes. Take a sample of the feed, retentate, and filtrate.

Analysis and Interpretation of Data

The percent passage is determined through the use of a UV visible spectrophotometer when testing with proteins, blue dextran, or latex beads; a differential refractometer is used when testing with PVPs or Bacitracin. Both instruments are calibrated with the solvent as the zero or base, and the initial recirculation solution as the 100% concentration.

1. Record the absorbance or refractive index of the initial recirculation solution.
2. Prepare at least 1 or 2 dilutions (50%, 25%) and measure the absorbance or refractive index.
3. Prepare a curve of concentration versus absorption (or refractive index).
4. Confirm linearity.
5. Measure the absorbance or refractive index of the collected filtrate samples.
6. Determine the percent passage using the following formula:

\[ \text{% Passage} = \left( \frac{\text{absorption of collected filtrate sample}}{\text{absorption of initial recirculation solution}} \right) \times 100 \]

The value can be read directly from the standard curve (Figure 23).

![Figure 23: Example of Curve of Concentration versus Absorption (Refractive Index)](image-url)
9 Detection Methods

9.1 Detection Methods for Cleaning Agents

9.1.1 Sodium Hydroxide
Residual sodium hydroxide can be determined by measuring the pH of effluent from the cassette and comparing the pH to that of the influent. When the two match, residual sodium hydroxide has been removed. The pH is a direct measure of the hydroxyl ion concentration and can be used to calculate residual hydroxyl ion (Table 24).

Table 24: Relationship of pH to Hydroxyl Ion Molar Concentration

<table>
<thead>
<tr>
<th>pH</th>
<th>Hydroxyl Ion Molar Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1x10^-7</td>
</tr>
<tr>
<td>8</td>
<td>1x10^-6</td>
</tr>
<tr>
<td>9</td>
<td>1x10^-5</td>
</tr>
<tr>
<td>10</td>
<td>1x10^-4</td>
</tr>
<tr>
<td>11</td>
<td>1x10^-3</td>
</tr>
<tr>
<td>12</td>
<td>1x10^-2</td>
</tr>
<tr>
<td>13</td>
<td>1x10^-1</td>
</tr>
<tr>
<td>14</td>
<td>1.0</td>
</tr>
</tbody>
</table>

9.2 Detection Methods for Storage Agents

9.2.1 Materials and Equipment
• Spectrophotometer
• Chromatograph
• DI water
• 50 mL sample vials
• HCL (5% solution)
• Ferric chloride (1% solution)

9.2.2 Sodium Azide
This method incorporates a colorimetric assay based on the reaction of sodium azide with ferric chloride at low pH. Sensitivity of the method is 1 ppm.

9.2.3 Sample Preparation
Prepare samples following these steps:
1. Prepare 25 mL each of sodium azide standards at concentrations of 50 ppm and 5 ppm and a DI water blank in clean 50 mL sample vials.
2. Transfer 25 mL of filtrate and retentate sample aliquots into clean 50 ml sample vials. Add 5 drops of 5% HCl to each sample vial.
3. Add 3 mL of 1% ferric chloride to each sample vial and mix. Let the samples stand for 15 minutes before measuring absorption.

9.2.4 Sodium Azide Detection
Follow these steps to determine the sodium azide concentration:
1. Set a spectrophotometer to 462 nm wavelength and set to "absorption" mode. Using the blank, set the absorption to zero.
2. Read and record the absorption value of the 0.005% (50 ppm) and 0.0005% (5 ppm) sodium azide standard solutions at 462 nm.
Note: At this operating condition, the working range of detection is 0.0002 – 0.0050% (2 – 50 ppm) sodium azide. For example, a sample containing 0.05% sodium azide would require about a 1/50 dilution to be in the working range of this detection test.

3. Prepare a graph of absorbance at 462 nm versus sodium azide concentration for the standards (Figure 24).

![Sodium Azide Concentration Determination](image)

Figure 24: Absorbance Graph

4. Read the samples on the spectrophotometer at 462 nm.
5. The sodium azide concentration is determined using the graph of absorbance.

9.2.5 Glycerin

The following steps describe the size exclusion chromatography method for determination of glycerin concentration. The sensitivity of the method is 1 ppm.

1. Prepare 100 ppm, 10 ppm, and 1 ppm glycerin standards.
2. Inject 50 μL of the glycerin standards on a size exclusion chromatography column (TOSO Haas, G3000PWXL) connected to an HPLC system (Hewlett Packard, Model #1050) using a refractive index detector (mobile phase: H₂O). Identify the glycerin peak and calculate the peak area for each standard. Prepare a standard curve of peak area versus glycerin concentration.
3. Inject 50 μL of each of the retentate and filtrate samples collected. Identify the glycerin peak found in each of the retentate and filtrate samples. Measure the peak area and determine glycerin concentrations from the standard curve.

9.3 Endotoxin Assay Procedure — ThermoMax Chromogenic Assay

This section describes the test procedure for detecting endotoxin in solution using Limulus Amoebocyte Lysate reagent incorporated into the THERMOmax Chromogenic (Endochrome* K) Assay.

Endochrome is a trademark of Charles River Laboratories.

9.3.1 Introduction

Recent advances in computer technology have made kinetic quantitation the method of choice for simultaneous measurement of endotoxin in multiple samples. This procedure provides a standard method for the use of the Molecular Devices THERMOmax microtiter plate reader with endotoxin standards in detecting and quantitating endotoxin in control and unknown samples.

9.3.2 Detection Limit

The detection limit is based on the sensitivity of the lysate being used. Sensitivity of 0.005 EU/mL can be achieved. This method is at least an order of magnitude more sensitive than the gel-clot procedure.

9.3.3 Materials

- Vortex mixer
- Falcon dilution tubes (no. 2057 or equivalent)
- Falcon 96 well microtiter plate (no. 3072)
• 1 and 5 mL pipettes
• 100 mL pipette with tips
• Eppendorf repeater pipette with 0.5 and 5 mL Combitips
• 37 °C heat block
• Chromogenic Kit (Endochrome K)
• WFI
• Endosafe* Control Standard Endotoxin (1000 EU/mL)
• ThermoMax with SoftMax* software (Version 2.34)

* SoftMax is a trademark of Molecular Devices, Inc.
Endosafe is a trademark of Dow Chemical Company.

9.3.4 Reagents
Control Standard Endotoxin (CSE)
500 ng in 5 mL = 1000 EU/mL.
Using a pipette, reconstitute with 5.0 mL of WFI directly into the vial. Close the vial, cover with aluminum foil, and invert in vortex. Tape the vial into position. Vortex the control sample for five minutes.

9.3.5 Endochrome* Reagent
Collect LAL powder into the bottom of its vial by tapping it on a firm surface. Unseal and release the vacuum by slowly lifting the stopper. Using a pipette and the 3.2 mL of LAL water provided with the LAL powder, rehydrate the LAL in its vial just before use. Gently swirl the vial until the LAL dissolves into a colorless solution. Discard the reagent if the seal is broken or color/opacity is present after rehydration. Store hydrated LAL reagent on ice while on the bench. Unused LAL may be frozen and thawed once.

Dilution of Control Endotoxin for Standard Curve Label dilution tubes: 500, 50, 5, 0.5, 0.05 EU/mL (including 0.005 EU/mL for Endochrome). Prepare dilution of endotoxin as described in Table 25. Vortex each dilution for one minute.

Table 25: Dilution of Control Endotoxin for Standard Curve

<table>
<thead>
<tr>
<th>To prepare</th>
<th>Take (X) mL of solution containing specified EU/mL</th>
<th>Dilute with (X) mL of WFI</th>
<th>Volume after dilution (mL)</th>
<th>Endotoxin concentration (EU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted Stock Solution</td>
<td></td>
<td>5.0</td>
<td>5.0</td>
<td>1000</td>
</tr>
<tr>
<td>Dilution 1</td>
<td>1.0 of 1000 EU/mL</td>
<td>1.0</td>
<td>2.0</td>
<td>500</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>0.1 of 500 EU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>50</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>0.1 of 50 EU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>5</td>
</tr>
<tr>
<td>Dilution 4</td>
<td>0.1 of 5.0 EU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Dilution 5</td>
<td>0.1 of 0.5 EU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Dilution 6</td>
<td>0.1 of 0.05 EU/mL</td>
<td>0.9</td>
<td>1.0</td>
<td>0.005</td>
</tr>
</tbody>
</table>

9.3.6 ThermoMax Setup
1. Turn on the instrument power switch. Click on SOFTMAX FOR WINDOWS, then the SOFTMAX icon.
2. Click on CONTROL function and drag to OPEN DRAWER.
3. Click on CONTROL function and drag to INCUBATOR, then activate.
4. Click on SET UP function and drag to INSTRUMENT and select the following:
   (i) Select KINETIC L1 under MODE
   (ii) Select wavelength 340 nm for TURBIDIMETRIC
   (iii) Select wavelength 405 nm for CHROMOGENIC
   (iv) Select Automix: ONCE
   (v) Set: 1-hour run time
   (vi) Set Read Interval: 00:14 sec
5. Click on SET UP function and drag to ANALYSIS
(i) Select Onset Time (sec)
(ii) Select Std Curve Fit: (log-log)
(iii) Select Spike Recovery Analysis
(iv) Select onset OD: 0.05
(v) Select onset limit: 0.2

6 Click on SET UP function and drag to TEMPLATE
7 Click and drag to select wells for:
   (i) Blanks
   (ii) Standard curve (0.05–5 EU/mL)
   (iii) Unknown + dilution
   (iv) Spike (0.5 EU/ml)

9.3.7 LAL Assay

Follow these steps to complete the LAL assay:
1. Place microtiter plate with lid on 37 °C heating block.
2. Remove lid and fill plate in duplicate with 100 μL of each:
   (i) Blank (WFI)
   (ii) 0.005 EU/mL standard
   (iii) 0.05 EU/mL standard
   (iv) 0.5 EU/mL standard
   (v) 5.0 EU/mL standard
   (vi) Unknown sample
   (vii) PPC = Unknown sample + 0.5 EU/mL
   (viii)(10 μL of 5.0 EU/mL of CSE standard)
   (ix) Water flush (negative control)
3. Incubate plate with lid on for 10 minutes at 37 °C.
4. After incubation, place 100 μL of LAL reagent into each well within 2 minutes using a repeater pipette.
5. Click on CONTROL function and drag to READ PLATE.
6. Place plate in reader without the lid.

9.3.8 Interpreting Results

The LAL assay results are considered accurate if the determination meets the following criterion:
1. Linearity of the standard curve within the concentration range used must be verified. No less than 3 endotoxin standards, spanning the desired concentration range, should be assayed in duplicate. The coefficient of correlation shall be greater than or equal to the absolute value of 0.98 for the determination to be valid.
2. Each unknown sample must be accompanied by the corresponding spike or positive product control (PPC). The mean endotoxin concentration of the PPC must be within ±50% of the corresponding standard curve concentration.

9.3.9 Analyst Qualification

Run the appropriate standard curve in triplicate, performing regression analysis on each individual point (r > 0.98) by selecting standard values individually (i.e., Std 1, Std 2 and Std 3 = 0.05 EU/mL). The standard curve for KTA LAL is three point (2 log) including points: 0.05, 0.5 and 5.0 EU/mL and for endochrome-K is four point (3 log) including points: 0.005, 0.05, 0.5, and 5.0 EU/ml.

The correlation coefficient must be greater than 0.98 to be acceptable for validation.
Repeat until the validation is acceptable. (Must be done with both Chromogenic Kinetic and Turbidometric Kinetic Assay to be validated on both procedures.)
10 Biosafety Test Results

Following are the report references for biological safety testing on Omega Membrane Cassettes.

10.1 Test Results — Biological Test for Plastics

Figure 25: Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Cover)
TOXIKON PROJECT NUMBER: 88G-0156

USP XXI BIOLOGICAL TEST FOR PLASTICS—CLASS VI

1.0 SUMMARY
Based on standards set by the study protocol, extracts of the test material did not produce a biological response when tested in laboratory animals.

2.0 PURPOSE
The purpose of the test was to screen solutions and test material extractions for potential toxic effects. The study tests the reaction of living animal tissue and normal animals to the presence of the test material and/or extracts of it.

3.0 REFERENCES
The test was conducted in accordance to the standards set by the USP XXI, National Formulary XVI, 1985, pages 1235-1237.

4.0 MANAGEMENT OF THE STUDY

4.1 Sponsor
Name and Address: Filtron Technology
500 Main Street
P.O. Box 119
Clinton, MA 01510

Project Officer: Attila Herczeg

4.2 Testing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

4.3 Toxikon Corporation's Supervisory Personnel Assigned to the Study:

Study Director: Herman S. Lilja, Ph.D., D.A.B.T.
Study Supervisor: Amy Austin, B.A.
Quality Assurance: Nancy DiGiulio, B.S.

5.0 COMPLIANCE
The present study conformed to all applicable laws and regulations. Specific regulatory requirements include U.S. FDA Good Laboratory Practice for Non-Clinical Laboratory Studies, 21 CFR, Part 58, 1979; "Guide for the Care and Use of Laboratory Animals" NIH Guide Supplement for Grants and Contracts, Volume 14 June 25, 1985; the "Laboratory Animal Welfare Act" (Public Law 89-544) as amended "Animals Welfare Act of 1970" (Public Law 91-579); and other applicable Department of Agriculture regulations and standards.

6.0 TEST SUBSTANCE
The following information was supplied by the Sponsor wherever applicable; it does not apply to confidential information. The sponsor was responsible for all test article characterization data as specified in 21 CFR, Part 58, 1979.
TOXIKON PROJECT NUMBER: 88G-0156

Description of the Test Substance: Screen Channel Filtration Cassette
Chemical/Common/Trade Name: Omega Cassette
Molecular Formula: N/A
Composition/Purity: Polyurethane Encapsulant, Polyester Screen, Omega Membrane

10.0 RESULTS

**Systemic Injection Test** (Table I and II)
Body Weights: All test animals exhibited weight gain.
Clinical Observations: None of the test or control animals showed overt signs of toxicity at any observation point.
Conclusion: The test sample extracts meet the requirements of the Systemic Test for Class VI Plastics (USP XXI, 1985).

**Intracutaneous Test** (Table III)
Body Weights: Four of the eight test animals exhibited a gain in body weight. Five test animals exhibited no weight change.
Clinical Observations: There were no skin reactions noted in any of the test animals. No signs of toxicity were observed.
Conclusion: The test sample meets the requirements of the Intracutaneous Test for Class VI Plastics (USP XXI, 1985). 

**Muscle Implant** (Table III)
Body Weights: One test animal exhibited a gain in body weight.

One of the test animals exhibited no weight change.

Clinical Observation: There were no signs of toxicity. The tissue surrounding the test material and the USP negative control strips appeared free of film, hemorrhage, and encapsulation.

Conclusion: The test material is considered non-toxic according to the procedures outlined in USP XXI for the Biological Test for Plastics, Class VI 1985.

13.0 AUTHORIZED SIGNATURE

[Signature]
Herman S. Lijja, Ph.D., D.A.B.T.
Study Director

8/9/80

Figure 27: Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Results)
## TABLE I
**USP XXI Biological Class Test**
Animal Examination Data Summary

<table>
<thead>
<tr>
<th>Test Group</th>
<th># of Animals and Sex</th>
<th>07/26/88 Day 0</th>
<th>07/29/88 Day 3</th>
<th>Signs of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>5 Females</td>
<td>19.7±2.0</td>
<td>21.6±1.3</td>
<td>0</td>
</tr>
<tr>
<td>1 in 20 Alcohol in Sodium Chloride</td>
<td>5 Females</td>
<td>21.4±1.3</td>
<td>23.2±1.3</td>
<td>0</td>
</tr>
<tr>
<td>Cottonseed Oil</td>
<td>5 Females</td>
<td>23.0±0.0</td>
<td>24.9±0.2</td>
<td>0</td>
</tr>
<tr>
<td>Polyethylene Glycol 400</td>
<td>5 Females</td>
<td>22.7±0.4</td>
<td>24.3±0.8</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 28: Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table I)

## TABLE II
**USP XXI Biological Class Test**
Animal Examination Data Summary

<table>
<thead>
<tr>
<th>Control Group</th>
<th># of Animals and Sex</th>
<th>07/26/88 Day 0</th>
<th>07/29/88 Day 3</th>
<th>Signs of Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride</td>
<td>5 Females</td>
<td>20.1±0.8</td>
<td>21.8±0.8</td>
<td>0</td>
</tr>
<tr>
<td>1 in 20 Alcohol in Sodium Chloride</td>
<td>5 Females</td>
<td>21.3±0.6</td>
<td>23.1±0.6</td>
<td>0</td>
</tr>
<tr>
<td>Cottonseed Oil</td>
<td>5 Females</td>
<td>21.5±0.5</td>
<td>23.3±0.6</td>
<td>0</td>
</tr>
<tr>
<td>Glycol 400</td>
<td>5 Females</td>
<td>20.7±0.5</td>
<td>22.4±0.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 29: Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table II)
### Table III
**USP XXI Biological Class Test**

**Animal Examination Data Summary**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Test Group</th>
<th>07/26/88 Day 0</th>
<th>07/29/88 Day 3</th>
<th>Skin Reaction</th>
<th>Edema</th>
</tr>
</thead>
<tbody>
<tr>
<td>8121</td>
<td>Sodium Chloride</td>
<td>2.80</td>
<td>2.80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8122</td>
<td>Sodium Chloride</td>
<td>2.60</td>
<td>2.62</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8123</td>
<td>1 in 20 Alcohol in Sodium Chloride</td>
<td>2.85</td>
<td>2.85</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8124</td>
<td>1 in 20 Alcohol in Sodium Chloride</td>
<td>2.79</td>
<td>2.80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8125</td>
<td>Cottonseed Oil</td>
<td>2.86</td>
<td>2.88</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8126</td>
<td>Cottonseed Oil</td>
<td>2.90</td>
<td>2.90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8127</td>
<td>Polyethylene Glycol 400</td>
<td>2.70</td>
<td>2.71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8128</td>
<td>Polyethylene Glycol 400</td>
<td>3.00</td>
<td>3.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>Test Group mean</td>
<td>2.81</td>
<td>2.82</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Implant Rabbits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8129</td>
<td>Implant</td>
<td>2.99</td>
<td>3.00</td>
<td>No abnormalities</td>
<td></td>
</tr>
<tr>
<td>8130</td>
<td>Implant</td>
<td>3.00</td>
<td>3.00</td>
<td>No abnormalities</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3.00</td>
<td>3.00</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 30: Results of USP XXI Biological Test for Plastics, In Vivo Class VI (Table III)
10.2 Test Results — Cytotoxicity Test

Figure 31: Results of L-929-MEM Cytotoxicity Test (Cover)
1.0 SUMMARY
The test material, Omega Cassette, was evaluated for biocompatibility using L929 mouse fibroblast cells. The test material extract exhibited no cytotoxic effects compared to the control extracts.

2.0 PURPOSE
To evaluate the biocompatibility of a test material using L929 mouse fibroblast cells. The test article extract was prepared in growth support medium and this medium was then used to replace the maintenance medium of confluent cell cultures. The results of the test were evaluated microscopically along with both a positive and negative control. The test material is reported in terms of its cytotoxic effects with respect to the controls.

3.0 MANAGEMENT OF THE STUDY

3.1 Sponsor
Name and Address: Filtron Technology
500 Main Street
P.O. Box 119
Clinton, MA 01510

Project Officer: Attila Herczeg

3.2 Testing Laboratory
Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

3.3 Toxikon Corporation's Supervisory Personnel Assigned to the Study:
Study Director: Laxman S. Desai D.Sc.
Study Supervisor: Asha Raghupathy M.S.
Quality Assurance: Nancy DiGiulio B.S.

4.0 COMPLIANCE
The present study conformed to all applicable laws and regulations. Specific regulatory requirements include FDA Good Laboratory Practice Guidelines 21 CFR, Part 58, 1979.

5.0 TEST SUBSTANCE
The following information was supplied by the Sponsor. The Sponsor was responsible for all test article characterization data as specified in 21 CFR, Part 58, 1979.

Description of the Test Substance: Screen Channel Filtration Cassette
Chemical/Common/Trade Name: Omega Cassette
Molecular Formula: Polyurethane Encapsulant, Polyester Screen, Omega Membrane
9.0 RESULTS
The test cultures were scored on the following:

0 = No observable change
1 = Slight but significant change in shape or loss of elongated cytoplasmic extensions. Some granulation. No loss in cell density.
2 = Major loss of cell shape, rounding of cells with general increase in granulation. Some loss of cell density.
3 = Significant loss of cell shape, much granulation of the cytoplasm. Over 50% of the cells have fallen from the culture tube surface.
4 = All cells are killed so that they have lysed or fallen from the culture tube surface.

<table>
<thead>
<tr>
<th>Culture Dishes</th>
<th>Duplicate A</th>
<th>Samples B</th>
<th>Neg Control</th>
<th>Pos Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Time</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24 Hours</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>48 Hours</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

10.0 DISCUSSION
The cells exposed to the test material extract did not exhibit morphological changes. The Negative control likewise did not exhibit any cellular changes. The positive control exhibited cell death at the 48 hour observation period. Based on the results of the test and control extracts, the test material is therefore considered non-cytotoxic.

14.0 AUTHORIZED SIGNATURE

Laxman S. Desai, D.Sc.
Study Director

Date: 8-22-5Y

Figure 33: Results of L-929- MEM Cytotoxicity Test (Results)
10.3 Test Results — Hemolysis

HEMOLYSIS TEST-DIRECT CONTACT WITH RABBIT BLOOD

PROJECT NUMBER: 88G-0163

Performed for

Filtron Technology
500 Main Street
P.O. Box 119
Clinton, MA 01510

Date Study Submitted
08/19/88

Study Director
Laxman S. Desai, D.Sc.

225 Wildwood Avenue, Woburn, Massachusetts 01801
(617) 933-6903, Telex 924-441, FAX 617-933-9196

Figure 34: Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Cover)
TOXIKON PROJECT NUMBER: 88G-0163

HEMOLYSIS TEST—DIRECT CONTACT WITH RABBIT BLOOD

1.0 PURPOSE
This test was used to determine whether the test material caused hemolytic activity when in direct contact with rabbit blood. The test article was tested with rabbit blood following the AuTian method.

2.0 SUMMARY
According to the procedures outlined in this test report, the test material is considered non-hemolytic since the calculated percentage of hemolysis was 3.2%.

3.0 MANAGEMENT OF THE STUDY

3.1 Sponsor
Name and Address: Filtron Technology
500 Main Street
P.O. Box 199
Clinton, MA 01510

Project Officer: Attila Herczeg

3.2 Testing Laboratory
Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

3.3 Toxikon Corporation's Supervisory Personnel Assigned to the Study:

Study Director: Laxman S. Desai, D.Sc.
Study Supervisor: Asha Raghupathy, M.S.
Quality Assurance: Nancy DiGiulio, B.S.

4.0 COMPLIANCE
The present study conformed to all applicable laws and regulations. Specific regulatory requirements include FDA Good Laboratory guidelines, 21 CFR, Part 58, 1979.

5.0 TEST SUBSTANCE
The following information was supplied by the Sponsor.

Description of the Test Substance: Screen Channel Filtration Cassette
Chemical/Common/Trade Name: Omega Cassette
Composition/Purity: Polyurethane Encapsulant, Polyester Screen, Omega Membrane

9.0 RESULTS

9.1 Tube #1 average absorbance = 3.4%
Tube #2 average absorbance = 3.1%
Tube #3 average absorbance = 3.0%
Positive Control average absorbance = 0.923
Negative Control average absorbance = 0.031
Average Value = 3.2% hemolysis

Figure 35: Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Summary and Results)
Figure 36: Results of the Hemolysis Test — Direct Contact with Rabbit Blood (Verification)
10.4 Test Results — Endotoxin Levels and Total Oxidizable Substances

TOXIKON PROJECT NUMBER: 94G-2269
LIMULUS AMEBOCYTE LYSATE AND TOTAL OXIDIZABLE SUBSTANCES

1.0 SUMMARY

A bacterial endotoxin level of 0.018 Eu/mL was eluted from the test article, Omega 100K Screen Channel, after the appropriate rinsing and cleaning procedures. The test article filtrate had undetectable levels of oxidizable substances.

2.0 PURPOSE

The objective of this study is to determine the amount of bacterial endotoxins and oxidizable substances that may be eluted from a filter material after the appropriate rinsing and cleaning procedure. The LAL test system utilizes a co-lyophilized mixture of Limulus Ameocyte Lysate (LAL), obtained from aqueous extracts of the circulating amebocytes of the horseshoe crab *Limulus polyphemus* and a synthetic color producing substrate to detect endotoxin chromogenically. Oxidizable substances were determined utilizing a colorimetric procedure.

3.0 MANAGEMENT OF THE STUDY

3.1 Sponsor: Filtron Technology Corporation
50 Bearfoot Road
Northborough, MA 01532

Project Officer: Diana Ricker

3.2 Testing Laboratory: Toxikon Corporation
225 Wildwood Avenue
Woburn, MA 01801

Study Director: Joseph A. Prezioso, Ph.D.
Study Supervisor: Stephanie Ferrante, M.S.
Quality Assurance: Katherine O’Kelly, B.S.

5.0 COMPLIANCE

The present study conformed to all applicable laws and regulations. Specific regulatory requirements will include the current Good Laboratory Practice for Nonclinical Laboratory Studies, FDA, 21 CFR, Part 58.

6.0 TEST AND CONTROL ARTICLES

6.1 The following information was supplied by the Sponsor wherever applicable. Confidential information did not apply. The Sponsor is responsible for all test article characterization and stability data as specified in the GLP regulations.

Test Article: Omega 100K Screen Channel
Lot/Batch #: 34180111
CAS/Code #: Not Supplied by Sponsor (N/S)
Physical State: Solid
Color: White
Density: N/S
pH: N/S
Stability: N/S
Solubility: N/S
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions
10.2 OXIDIZABLE SUBSTANCES ASSAY

10.2.1 The test article filtrate meets the requirement for the test, if the pink color of the solution does not disappear completely.

11.0 RESULTS

11.1 LAL ASSAY

The standard curve met the criteria for linearity by having a correlation coefficient of -0.994. The positive product control contained 0.574 Eu/mL which is 14.8% above the 0.5 Eu/mL standard indicating no inhibition or enhancement was present. The test article filter had an endotoxin concentration of 0.018 Eu/mL.

11.2 OXIDIZABLE SUBSTANCES ASSAY

The test article filtrate did not cause the pink color of the solution to disappear completely.

12.0 CONCLUSION

A bacterial endotoxin level of 0.018 Eu/mL was eluted from the test article, Omega 100K Screen Channel, after the appropriate rinsing and cleaning procedures. The test article filtrate had undetectable levels of oxidizable substances.

13.0 CONFIDENTIALITY STATEMENT

Statements of confidentiality were as agreed upon prior to study contract initiation.

14.0 RECORDS

<table>
<thead>
<tr>
<th>Original Data:</th>
<th>Toxikon Corporation Archives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Report:</td>
<td>Toxikon Corporation Archives</td>
</tr>
<tr>
<td>Test Article:</td>
<td>Remaining test article will be disposed.</td>
</tr>
</tbody>
</table>

15.0 VERIFICATION DATA

<table>
<thead>
<tr>
<th>Protocol Signature (Toxikon):</th>
<th>10/21/94</th>
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<tr>
<td>Project Log Date:</td>
<td>11/30/94</td>
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<td>12/01/94</td>
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<td>Technical Completion:</td>
<td>12/07/94</td>
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<tr>
<td>Final Report:</td>
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</tr>
</tbody>
</table>

16.0 AUTHORIZED SIGNATURES

**Joseph A. Prezioso, Ph.D.**

Study Director

Date: 12/07/94

Figure 37: Endotoxin Levels and Total Oxidizable Substances (Conclusions and Verification)

Endnotes

Notice

All data, specifications and information contained in this publication are based on information we believe reliable and represent values in effect at the time of printing. Pall Corporation reserves the right to make changes without prior notice. USTR 2143  Rev B