Validation Guide
Clipster® Aseptic Disconnector
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1. Introduction

Sartorius Stedim Biotech bags and systems are widely used in biopharmaceutical processes for a variety of unit operations of the commercial production of drug products such as vaccines, recombinant proteins and monoclonal antibodies and for the development of future biomolecules in clinical phases.

Buffers and media are increasingly formulated, sterile filtered and stored in single-use Fluid Management Systems (FMS) that involve Flexel® and Flexboy® Bags integrated with filters, impeller mixers, tubing, connectors and monitoring tools. Product intermediates are also filtered and stored between UF/DF and chromatography purification steps in Gamma sterile Fluid Management Systems.

Fluid Management Systems are also adopted for the formulation, filtration and aseptic processing of final drug products.

From buffer and media preparation, cell culture operations, purification operations up to final formulation, filtration and transfer, the aseptic disconnection is a key element for the successful implementation of disposable manufacturing processes. The Clipster® Aseptic Disconnector allows an aseptic disconnection of Silicone (Pt) tubing in a nonclassified and classified environments.

The Clipster® Aseptic Disconnector is qualified, manufactured and released according to stringent product validation protocols and Quality Control testing to offer safe and robust single-use processes to the end users of the Biopharmaceutical industry.

Validation, as used in these guidelines, comprises the systematic testing of essential production steps and equipment in the R&D and production departments, including testing and inspection of final products with the goal of ensuring that the finished products can be reliably and reproducibly manufactured in the respect with the established production and quality control procedures.

We have compiled this validation guide so that users of Clipster® Aseptic Disconnector can plan, implement and document their own validation procedures.

This validation guide is applicable for both Clipster® Aseptic Disconnector sold as a stand-alone product and pre-assembled on Sartorius Stedim Biotech bags.
1.1 Security of Supply
As a result of the broad adoption of Fluid Management Systems incorporating the Clipster® Aseptic Disconnector in critical process steps of commercial drug manufacturing, security of supply has become the major issue for our customers.

Sartorius Stedim Biotech offers the most secured manufacturing resources and capabilities on the market to ensure the strongest security of supply. We hold the most modern facilities in Europe, North America, North Africa for production of pharmaceutical and medical plastic Fluid Management Systems with a total of 4800 m² (48,000 sq ft) of ISO 7 clean room (Class 10,000 or Grade C).

Our manufacturing plants operate under a common information system in order to offer flexibility and reliable product transfer from one location to the other. Consistent process performance is ensured by the on-going qualification of all components and assemblies, manufacturing processes and personnel.

1.2 cGMP Quality Assurance from Sartorius Stedim Biotech
Consistent high quality of the Clipster® Aseptic Disconnector is assured by careful selection of the raw material, well-planned and validated production technologies and an exceptionally efficient Quality Assurance Department, all of which result in high batch-to-batch reproducibility.

1.3 Quality Assurance
For quality assurance, all materials are carefully selected and validated in accordance with in-house guidelines and the specifications of our R&D Department including product performance and security of supply. Documentation begins with the inspection of the incoming raw material including in-process material, molded parts, etc. for manufacture. The finished Clipster® Aseptic Disconnector components undergo quality controls. This involves 100% non-destructive testings of each individual component. A lot is not released until all in-process and final quality control data are available.

1.4 Complete Traceability
The type and lot number are printed on the label of the protective plastic bag and on the label of the box in which the Clipster® Aseptic Disconnector components are packed. The traceable lot number allows convenient retrieval of all data compiled on the materials used, production steps and QC tests.

1.5 Quality Management System
Sartorius Stedim Biotech implemented Quality Management Systems to assure consistent high quality of the Clipster® Aseptic Disconnector.

Exemplary Quality Systems Certificates

The complete Quality Systems Certificates are continuously updated and can be downloaded on our website: www.sartorius-stedim.com.
2. Technical Specifications

2.1 Type and Part Number Overview

The Clipster® Aseptic Disconnector size L is used for Silicone (Pt) tubing 3/8” ID × 5/8” OD and ½” ID × ¾” OD.

The Clipster® is composed of 4 components: 2 identical bottom components and 2 identical top components.

<table>
<thead>
<tr>
<th>Size</th>
<th>Part Number</th>
<th>Top Component</th>
<th>Bottom Component</th>
<th>ID-OD</th>
<th>Silicone (Pt) Tubing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>½ – ¾</td>
<td>TU100357</td>
</tr>
</tbody>
</table>

Note:
The Clipster® Aseptic Disconnector size L is validated only on the Si (Pt) listed in the above table.
2.2 Material of Construction

The Clipster® Aseptic Disconnector size L is a non contact fluid component.

**Connector Material:**
Polycarbonate

2.3 Mechanism of the Aseptic Disconnection

The aseptic disconnection is mechanically performed in a 4-step operation. The Clipster® is first assembled on the tube, then, placed into a tool that clamps the Clipster® and cuts the tube. Finally, the Clipster® is disconnected.

2.4 Sterilization Compatibility

The Clipster® Aseptic Disconnector may be unsterile when sold as a stand-alone product or sterile when sold pre-assembled on Sartorius Stedim Biotech bags.

**Sterilization Compatibility:**
Gamma Irradiation ≤ 50 kGy

The Clipster® Aseptic Disconnector is not validated for steam sterilization and ethylene oxide sterilization.

2.5 Shelf Life

The shelf life is certified in normal storage conditions, stored in their original packaging with overpouches and cartons and in a protected warehouse.

The shelf life for the Clipster® Aseptic Disconnector sold as a stand-alone product (not sterile):
3 years accelerated ageing validated (3 years natural ageing validation is in progress)

The shelf life for the Clipster® Aseptic Disconnector sold pre-assembled on Sartorius Stedim Biotech bags (sterile):
3 years accelerated ageing validated (3 years natural ageing validation is in progress)

The natural ageing shelf life statement will be updated respectively after receipt of the results at 1 year, 2 years and 3 years.

2.6 Dimensions

**Clipster® Aseptic Disconnector Top Component**

<table>
<thead>
<tr>
<th>Length [mm]</th>
<th>Height [mm]</th>
<th>Width [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
</tr>
<tr>
<td>19.3</td>
<td>19</td>
<td>34.0</td>
</tr>
</tbody>
</table>

**Clipster® Aseptic Disconnector Bottom Component**

<table>
<thead>
<tr>
<th>Length [mm]</th>
<th>Height [mm]</th>
<th>Width [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
</tr>
<tr>
<td>21.5</td>
<td>16.2</td>
<td>34.0</td>
</tr>
</tbody>
</table>

**Clipster® Aseptic Disconnector after Clamping**

<table>
<thead>
<tr>
<th>Length [mm]</th>
<th>Height [mm]</th>
<th>Width [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
</tr>
<tr>
<td>40.0</td>
<td>19.3</td>
<td>34.0</td>
</tr>
</tbody>
</table>
3. Functional Qualification Tests

The qualification of the Clipster® Aseptic Disconnector was performed according to Sartorius Stedim Biotech standard methods. The qualification tests were performed at ambient temperature after Gamma irradiation at 50 kGy which represents a worst case scenario. Therefore, all the test results are also applicable for the unsterile Clipster® Aseptic Disconnector.

The qualification tests were performed on the 2 disconnected Clipster® parts after the disconnection.

3.1 Functional Qualification Tests Overview

Clipster® Aseptic Disconnector
Pre-assembled on tube

Gamma irradiation 50 kGy

Visual Inspection

At $t_s$

Clamping tests

Aseptic disconnection

Aseptic disconnection

3 years ageing (accelerated)

After 6 month

Air leak

Tensile strength test

Colored water

Air leak

Tensile strength test
3.2 Sampling
The following components were used for the functionnal qualification tests:

**Connector samples:**
Description: Clipster® Aseptic Disconnector size L
Reference: 642-L
Lot number: G056010695

**Tube samples:**
Material: Silicone (Pt)
Dimensions [inch]: 3/8 ID x 5/8 OD
Reference: TU109871
Lot number #1: 01988A10
Lot number #2: 01774A09

Material: Silicone (Pt)
Dimensions [inch]: ½ ID x ¾ OD
Reference: TU100357
Lot number #1: 01849A10
Lot number #2: 03625A10

3.4 Clamping Test

**Purpose**
The Clipster® which are pre-assembled on Fluid Management bags and transfer lines must remain unclamped in order to allow the fluid transfer during processing.

The goal of the clamping test is to check that pre-assembled Clipster® will not mistakenly clamp the tubing during shipping and storage applications.

**Test Method**
The Clipster® is pre-assembled on the tube. After Gamma irradiation and visual inspection, the samples are positioned horizontally. A mass of 4kg is placed onto the Clipster® for 10 days to simulate a shipping and a storage worst case scenario where the weight of the bag assembly would be applied on the Clipster®.

The test is passed if after 10 days the Clipster® stays unclamped.

**Results**

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L + Si (Pt) 3/8 x 5/8</td>
<td>4</td>
<td>Pass</td>
</tr>
<tr>
<td>642-L + Si (Pt) ½ x ¾</td>
<td>4</td>
<td>Pass</td>
</tr>
</tbody>
</table>
3.5 Air Leak Test

Purpose and Test Method
The purpose of the air leak test is to check the clamping tightness after the tubing disconnection.
The Clipster® is pre-assembled on the tube. After Gamma irradiation and visual inspection, the Clipster® is disconnected at t₀ and after 3 years accelerated ageing.
The air leak test is performed on the disconnected Clipster® parts at t₀ and after 3 years accelerated ageing. The samples are submersed and a pressure of 0.5 bar and 1 bar are applied for 10 s.

The test is passed if no air bubble appears.
The air leak pressure limit is also measured.

Results at t₀

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>10</td>
<td>Pass</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>10</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Air leak pressure limit results at t₀

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>2</td>
<td>( P_{\text{limit}} &gt; 2.5 \text{ bar}^* )</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>2</td>
<td>( P_{\text{limit}} &gt; 2 \text{ bar}^* )</td>
</tr>
</tbody>
</table>

Results after 3 years accelerated ageing + 6 months after disconnection

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>15</td>
<td>Pass</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>15</td>
<td>Pass</td>
</tr>
</tbody>
</table>

Air leak pressure limit results after 3 years accelerated ageing + 6 months after disconnection

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>3</td>
<td>( P_{\text{limit}} &gt; 2.5 \text{ bar} )</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>3</td>
<td>( P_{\text{limit}} &gt; 2 \text{ bar} )</td>
</tr>
</tbody>
</table>

* \( P_{\text{limit}} \) is above the pressure limit resistance of the Si (Pt) tubing
3.6 Colored Water Leak Test

**Purpose and Test Method**

The purpose of the colored water leak test is to check the clamping tightness after the tubing disconnection when the tube is filled with liquid.

The Clipster® is pre-assembled on the tube. After Gamma irradiation and visual inspection, the tube is filled with colored water and plugged on both extremities. The tube is disconnected and immediately positioned vertically with the 2 disconnected Clipster® parts at the bottom position. Each sample is wiped with a white paper at the disconnection area.

The test is passed if no colored water leak appears on the white paper.

**Results**

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{3}{8} \times \frac{5}{8}$</td>
<td>8</td>
<td>Pass</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{1}{2} \times \frac{3}{4}$</td>
<td>8</td>
<td>Pass</td>
</tr>
</tbody>
</table>
3.7 Tensile Strength Test

Purpose and Test Method
The purpose of the tensile strength test is to check the assembly strength after the tubing disconnection.

The Clipster® is pre-assembled on the tube. After Gamma irradiation and visual inspection, the Clipster® is disconnected at \( t_0 \) and after 3 years accelerated ageing.

The tensile strength test is performed on the disconnected Clipster® parts at \( t_0 \) and after 3 years accelerated ageing.

Results at \( t_0 \)

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Limit</th>
<th>Measurements</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Max [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>4</td>
<td>( F &gt; 80 \text{N} )</td>
<td>112.7</td>
<td>108</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>4</td>
<td>( F &gt; 80 \text{N} )</td>
<td>137.4</td>
<td>128.8</td>
</tr>
</tbody>
</table>

Results after 3 years accelerated ageing + 6 months after disconnection

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Sample Quantity</th>
<th>Limit</th>
<th>Measurements</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Average [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Max [N]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Standard Deviation</td>
<td></td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{3}{8} \times \frac{5}{8} )</td>
<td>4</td>
<td>( F &gt; 80 \text{N} )</td>
<td>112.6</td>
<td>109.9</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) ( \frac{1}{2} \times \frac{3}{4} )</td>
<td>4</td>
<td>( F &gt; 80 \text{N} )</td>
<td>146.3</td>
<td>136</td>
</tr>
</tbody>
</table>
4. Bacterial Challenge Test

Purpose
The goal of this study is to demonstrate the microbial barrier property of the disconnected Clipster® Aseptic Disconnector.

This scheme is developed by Sartorius Stedim Biotech and conducted according to the ISO15747: "Plastic container for intravenous injection – section 4.3.1 Biologic requirements – microorganisms impermeability testing". The strain of Bacillus atropheus (former Bacillus subtilis) has been chosen according to the recommendations of this standard. The microbial challenge test has been performed under worst case conditions with regards to an aseptic disconnection performed with the Clipster®.

The study covers the microbial impermeability of the Silicone (Pt) tubing connected to a bag system after an aseptic disconnection performed with the Clipster®.

Sampling Description
Specific assembly has been developed to ease aseptic media fill.

All tubing/bag and Clipster® used for the study are Gamma irradiated at 50 kGy and accelerated aging (equivalent to 3 years) to perform the bacterial challenge test in worst case conditions.

Connector Samples
Description: Clipster® Aseptic Disconnector size L
Reference: 642-L
Lot number: G056010695

Tube Samples
Material: Silicone (Pt)
Dimensions [inch]: 3/8 ID x 5/8 OD
Reference: TU109871
Lot number: 02159A10

Material: Silicone (Pt)
Dimensions [inch]: ½ ID x ¾ OD
Reference: TU100357
Lot number: 03395A10
**Test Method**

First, the test samples, the positive and negative controls are filled aseptically under ISO5 laminar flow hood with the suitable volume of culture media (Soybean Casein peptone broth). The test samples, the positive and negative controls are thereafter submitted to a pre-incubation at 30–35 °C for 7 days. The absence of growth confirms the sterility of the test samples filled with media before the bacterial challenge test.

The challenge suspension is prepared with the Bacillus Atropheus strain ATCC # 9372 (ISO11138). A dilution is performed with WFI in order to obtain a suspension of not less than $1 \times 10^6$ CFU/mL. The concentration of the challenge suspension is confirmed by a microbial method.

The Clipster® is disconnected after 3 years accelerated ageing.

After the disconnection after 3 years accelerated ageing, each disconnected Clipster® part is immersed in the bacterial challenge solution for 30 minutes, dried under laminar flow hood and stored at ambiant temperature until the different due date: $T=0$ and 1 month. The validation is in progress for the due dates 2 months, 3 months and 6 months after disconnection.

At the end of each due date the disconnected Clipster® is incubated at 30–35 °C for 7 days.

Positive and negative controls are challenged in parallel to assess the final results.

One positive control per series for each due date is conducted to prove the fertility of the system. One mL of Bacillus Atropheus suspension at $10^6$ CFU/mL is injected with a sterile syringe throughout the port tube. The concentration of the positive control is confirmed by a microbial method. This positive control is representative of both sizes of silicone tubing.

One negative control per series is conducted to verify the aseptic filling conditions. This negative control is representative of both sizes of silicone tubing.
Sample preparation + Gamma Irradiation 50 kGy + Accelerated ageing

Aseptic filling of test samples with media Casein Peptone-Soybean

First incubation at 30–35 °C for 7 days

Aseptic disconnection

Control of microbial growth # 1 (to check aseptic filling)

Test assembly (disconnected) immersion in the bacterial suspension 10^6 CFU/mL for 30 min

Storage in PE pouch for different due date

Final incubation of the test assembly (disconnected) at 30–35 °C for 7 days

No microbial growth observed on Negative controls
No microbial growth observed on Test Samples
Positive microbial growth observed on Positive Control

Negative control

Positive control preparation with 10^2 CFU per bag
The concentration of the microbial challenge suspensions was confirmed at $2.4 \times 10^6$ and $1.3 \times 10^2$ CFU/mL for the liquid immersion test and the positive controls respectively. Microbial growth in the positive controls demonstrates the fertility of the suspension and the absence of inhibition phenomena from the test assembly.

The absence of growth for the tests assembly after the bacterial challenge immersion demonstrates the impermeability of the disconnected Clipster® to the Bacillus atropheus strain under the tested conditions.

The microbial growth results for positive, negative and test articles are in conformance with the ISO15747 "Plastic container for intravenous injection - section 4.3.1 Biologic requirements - microorganisms impermeability testing".

**Conclusion**

The microbial barrier property of the disconnected Clipster® has been validated up to 1 month after the aseptic disconnection by using the ingress testing method with worst case conditions.

The validation is in progress for the due dates 2 months, 3 months and 6 months after disconnection. The validation guide will be updated respectively after receipt of the results at 2 months, 3 months and 6 months after disconnection.

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**Results at t₀**

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{3}{8}$ x $\frac{5}{8}$</td>
<td>No growth</td>
</tr>
<tr>
<td>Positive control</td>
<td>Growth</td>
</tr>
<tr>
<td>Negative control</td>
<td>No growth</td>
</tr>
</tbody>
</table>

**Results after 3 years accelerated ageing at:**

- **Due date: T=0**

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{3}{8}$ x $\frac{5}{8}$</td>
<td>No growth</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{1}{2}$ x $\frac{3}{4}$</td>
<td>No growth</td>
</tr>
<tr>
<td>Positive control</td>
<td>Growth</td>
</tr>
<tr>
<td>Negative control</td>
<td>No growth</td>
</tr>
</tbody>
</table>

- **Due date: T=1month**

<table>
<thead>
<tr>
<th>Assembly</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{3}{8}$ x $\frac{5}{8}$</td>
<td>No growth</td>
</tr>
<tr>
<td>642-L (Disconnected) + Si (Pt) $\frac{1}{2}$ x $\frac{3}{4}$</td>
<td>No growth</td>
</tr>
<tr>
<td>Positive control</td>
<td>Growth</td>
</tr>
<tr>
<td>Negative control</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Some pre-tests have also been done by using the same method at t₀.
5. Chemical Resistance

The Clipster® Aseptic Disconnector is a non contact fluid component. In case of external long term contact of the Clipster® with a fluid, chemical resistance studies can be proposed upon customer’s request according to specific process conditions (i.e. type of solution, time, temperature).