



sartorius stedim  
biotech

Enhanced Sterility Assurance  
in Stopper processing

# White Paper

turning science into solutions

## Table of Contents

Abstract	3
Background	3
Study Design	4
Preliminary Validation	6
Aseptic Transfer Validation Method	7
Conclusion	10
Bibliography	11

# Enhanced Sterility Assurance

## in Stopper processing: A Unique System to Unload Stoppers from a Stopper Processing System

### Abstract

Stoppers for pharmaceutical primary packaging must preserve the safety and efficacy of injectable drugs. Thus, before their intended use, these stoppers undergo multiple processing steps, including washing, rinsing, siliconization, steam sterilization and drying. Each step is critical to ensure the physical and mechanical properties of the stoppers by reducing particles, adding the appropriate silicon volume, sterilizing and avoiding re-contamination due to residual moisture.

An additional critical step after the stopper processing is maintenance of closure integrity until filling in aseptic conditions.

This paper discusses the validation of the transfer of stoppers from a stopper processing system, using a new aseptic transfer technology. It demonstrates that this technology maintains the sterility of the stoppers after their transfer into sterile single-use bags in an ISO Class 7 or 8 environment.

### Background

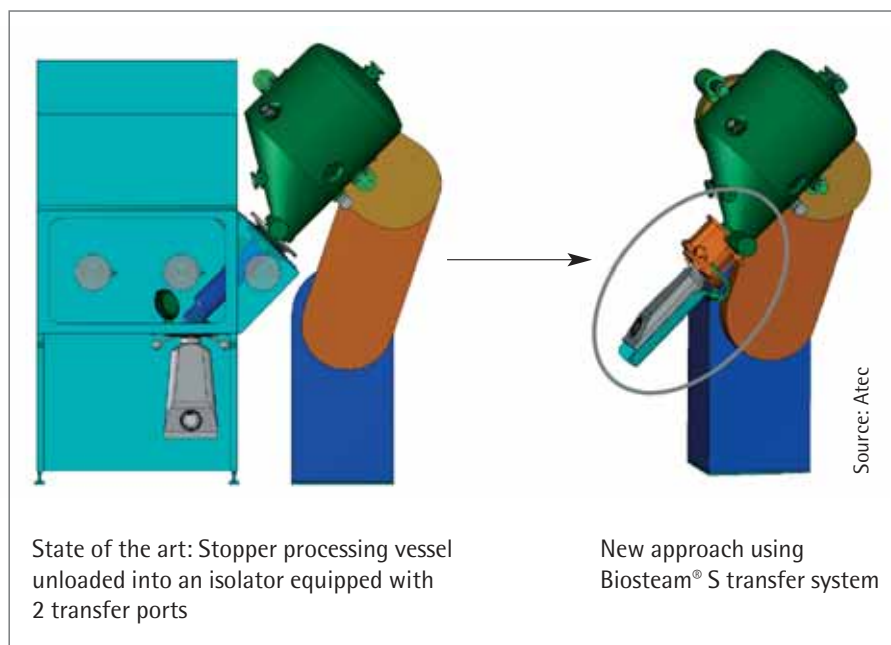
A number of industry trends are changing the drug development landscape. The increase in the production of biologicals, a market which is forecasted to reach \$12.6 billion in 2010 [1], along with live vaccines, large molecules and protein based injectable drugs, require advanced aseptic processing. Such growth has driven the need for new and improved filling technologies. Consequently, both EU & US regulatory authorities recommend using isolators for aseptic processing to decrease the risk of microbiological contamination due to human interaction [2].

Prior to the aseptic filling process, it is critical to maintain sterility assurance during stopper processing and transfer to filling in an ISO class 5 barrier systems [3].

This has led to a renaissance in industry trends towards novel rubber formulations, as well as new techniques for "ready-to-sterilize" and "ready-to-use" elastomeric components. In both cases, portions of the stopper processing are within the responsibility of the stopper manufacturer. Ready-to-sterilize (RTS) stoppers are packaged in steam sterilizable bags, streamlining manufacturing operations related to closure preparation. Regulatory agencies have promulgated strict requirements for qualification and validation processes, test method systems, and components [2]. Recently, the option of ready-to-use (RTU) stoppers has become available to pharmaceutical and biopharmaceutical companies. The RTU process has been developed to meet FDA validation requirements for equipment, processes, product, documentation and testing methods according to CFR 211.94 Drug product containers and closures [4]. Choosing RTU sterile components allows end-users to move packaged stoppers from their warehouse directly to the sterile fill area. However, the majority of RTU components are Gamma irradiated. Studies have shown the possibility of a negative impact on the elastomeric polymer chain caused by the Gamma rays [5].

Despite the trend towards using RTS and RTU stoppers, the control of the full stopper processing remains a reality and a preferred solution for many companies. The decision driver for in house stopper processing is the production quality (i.e. particle reduction, uniform siliconization, dryness and control of the sterilization process).

The existing method of unloading sterile stoppers from a stopper processing system is the use of an intermediate isolator for maintaining the sterility of the components. However, the Biosafe® Biosteam® S system allows the user the additional flexibility of processing in an ISO Class 7 or even 8 environment without requiring an intermediate isolator to ensure aseptic transfer (diagram 1).



**Diagram 1:** New approach for packaging sterile stoppers from a stopper processing vessel using new Biosafe® Biosteam® S transfer system

### Study Design

The new technique of sterile stopper transfer developed by Sartorius Stedim Biotech (SSB) is fully innovative and not yet described in ISO or US and European Pharmacopoeia. SSB developed this validation methodology according to the regulatory requirements applicable to the sterility testing, bioburden and sterilization process validation used in the pharmaceutical and biotechnology industries.

### Rationale

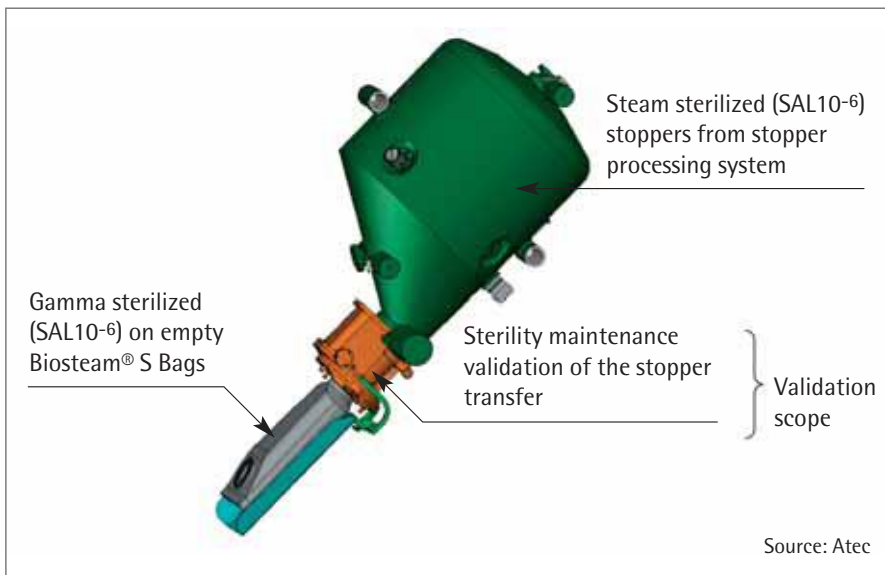
The definition of the sterility, as stated in the EP and ISO is "free of viable micro-organism" [6], [7]. Microbial inactivation is measured exponentially and the survival of one microorganism can be expressed in terms of probability. According to the regulatory requirements, to validate a sterilization process, this probability must be reduced to a very low number, as expressed by the Sterility Assurance Level (SAL). The sterilization process is considered validated if the demonstrated SAL is  $10^{-6}$ .

A sterilization process is defined by exposing products to a chemical agent or a physical treatment; it is therefore not applicable in the case of the Biosafe® Biosteam® S transfer operation. The following validation methodology demonstrates how the Sterility Assurance Level critical parameter is maintained using the proposed transfer method.

Pre requisites:

- Stoppers are steam sterilized; the sterilization process is validated to guarantee a SAL of  $10^{-6}$  [9].
- The disposable Biosteam® S Bag is sterilized by Gamma irradiation at a minimum 25 kGy. The sterilization process is validated according to NF EN ISO 11137 [10] to guarantee a SAL of  $10^{-6}$ .

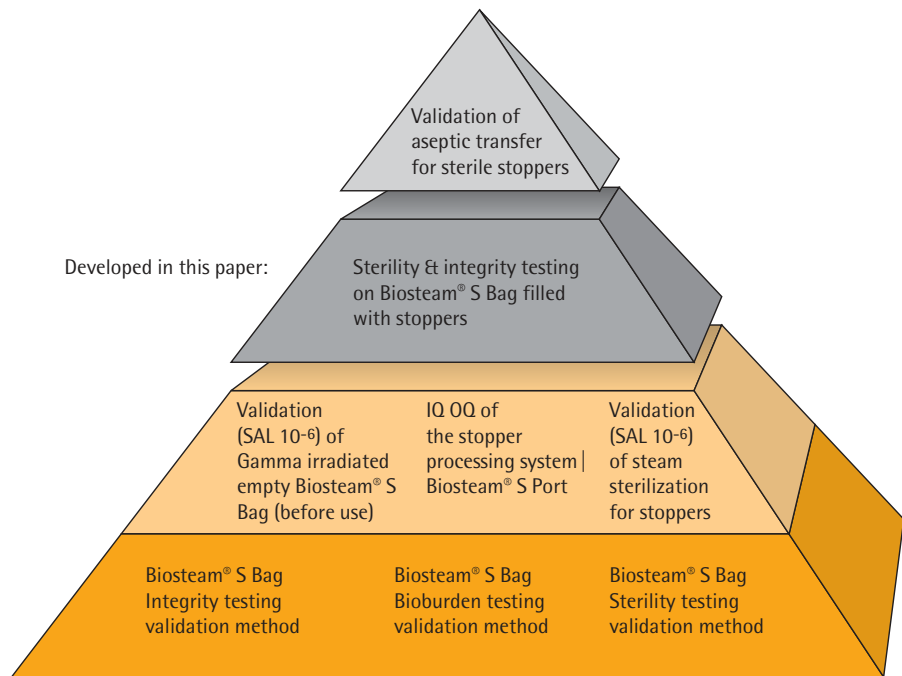
The objective of this validation study was to demonstrate the system integrity through multiple unloading processes and confirm the sterility status of stoppers within the Biosteam® S Bags filled with stoppers (diagram 2).



**Diagram 2:** Validation scope: Aseptic transfer of sterile stoppers

The mechanical and microbial qualification study for the stopper processing system (SPS) and the disposable Biosteam® S Bag ensures the integrity of the complete system before, during and after fractional unloading (up to 40 connections and disconnections).

Sterility tests performed on the disposable Biosteam® S Bag filled with stoppers (32 units sampled from 3 sterilization runs) prove the efficacy of multiple aseptic transfers.



**Diagram 3:** Global validation methodology

### **Preliminary Validation**

The global validation programs were performed as described in the Diagram 3. However, for simplification, the two following preliminary programs are not described in this paper:

- Installation Qualification (IQ) and Operational Qualification (OQ) of the stopper processing system | Biosafe® Biosteam® S Port.
- Stopper sterilization validation.

### **Validation of Testing Methods**

Validation of the bioburden and sterility testing methods conform to NF EN ISO11737-1 [6] and EP 2.6.1 [7] respectively:

- absence of inhibitory factors verified by bacteriostasis and fungistasis testing
- culture conditions in routine in conformance with the requirements
- a recovery coefficient conform to the specifications ( $\leq 2$ )
- sterility testing conditions are relevant and not inhibitory

The ammonia leak testing method performed on Biosteam® S Bags is comparable to microbial ingress testing according to ISO15747 - §4.3 [11].

### Validation of the Gamma Irradiation Sterilization of Biosteam® S Bags

The Biosteam® S Bags were validated according to NF EN ISO 11137 [10]  $VD_{max}^{25}$  method (selection method linked with bioburden less than 1000CFU per device) for Gamma sterilization using a minimum dose of 25 kGy and a maximum dose of 45 kGy in order to obtain a  $10^{-6}$  Sterility Assurance Level.

Bioburden testing was performed on 10 units from 3 lots, the average results were calculated and the verification dose  $VD_{max}^{25}$  (SAL  $10^{-2}$ ) was obtained.

The verification dose experiment was performed by exposing 10 items from one single batch to the  $VD_{max}^{25}$  irradiation dose. Sterility testing results met the requirement (no more than one positive) and therefore qualifies the minimum sterilization dose of 25 kGy. Product bioburden is also routinely verified to test for potential process variations. This testing allows for a comparison to be made between the product bioburden resistance and the resistance of the experimental population.

Dose audit verification is performed in order to confirm the validity of the 25 kGy minimum.

### Aseptic Transfer Validation Method

The goal of this validation was to demonstrate the sterility maintenance of stoppers once sterilized in bulk in a stopper processing vessel and packaged into Gamma irradiated Biosteam® S Bags.

To perform this validation work, the Biosafe® Biosteam® S Port was installed on a 250L process vessel of an ATEC stopper processing system. The stoppers used were liquid vial rubber stoppers with a dimension of  $\varnothing$  13 mm.

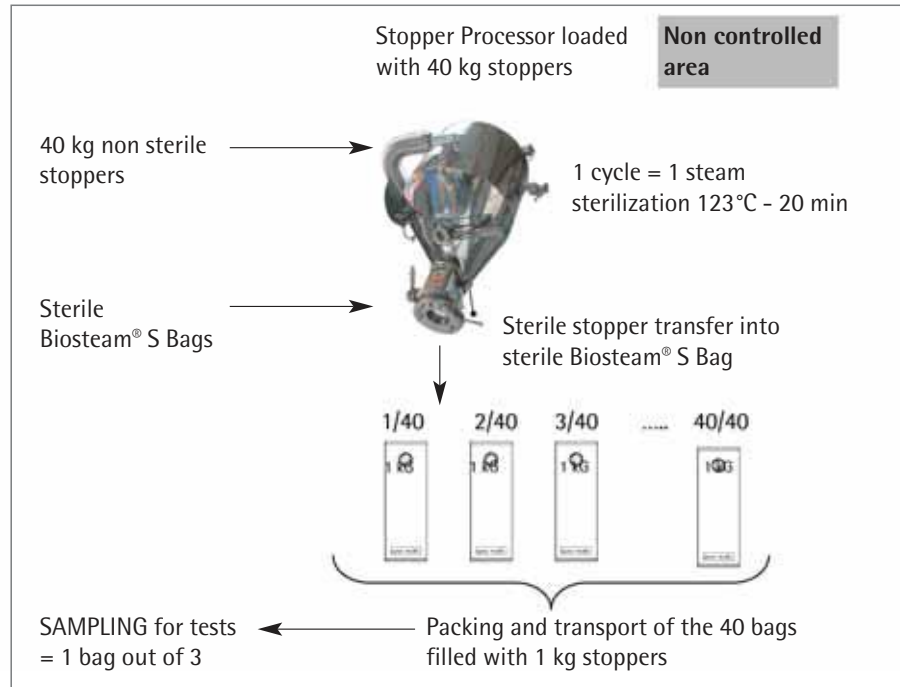
### Worse Case Rationale

The worse case situation described in the table below was chosen for the validation study according to the following criteria:

Criteria	Normal conditions	Worse case scenario selected for this validation
Number of sterilization runs	1 run per day	3 consecutive sterilization runs
Multiple unloading sequences (number of connections-disconnections)	10 connections-disconnections (*)	20 connections-disconnections & 40 connections-disconnections
Unloading environment	ISO Class 7 or 8 environment	Uncontrolled area
Port Decontamination	Sanitization with alcohol of port seals between each connection-disconnection	No port Sanitization
Storage Conditions	Individual storage on clean shelving in Class C or B	Packaged in boxes of 20 units, without any added protection between bags

(\*) Normal conditions include a 250L stopper processing vessel transferred into 10 units of 25L Biosteam® S Bags. As a worse case scenario, 20 and 40 connections-disconnections were challenged to determine partial filling of Biosteam® S Bags.

## Testing Conditions



**Diagram 4:** Validation of sterility & integrity testing on Biosteam® Bags filled with stoppers

As shown in the diagram 4, the validation study consisted in the following steps:

- Three steam sterilization runs were performed in 250L Atec stopper processing vessel at 123°C for 20 min.
- Each sterilization load was completed with 40 kg of Ø 13 mm stoppers.
- In grey zone, unloading of 1 kg of stoppers in one Biosteam® S Bag after 20 to 40 connections-disconnections.
- No port decontamination with alcohol performed between each connection during the tests.
- Biosteam® S Bags filled with 1 kg of stoppers and packed 20 units per box.
- Biosteam® S Bags were transported by truck to the laboratory.
- Biosteam® S Bags filled with 1 kg of stoppers were sampled 1 out of 3 for integrity and sterility testing.



## Bag Integrity Test

**Objective:** Packaging integrity testing by chemical gas detection

**Sampling:** 1 out of 3 Biosteam® S Bags filled with 1 kg of stoppers (a total of 35 units) were tested for integrity.

**Method:** To detect a leak, the Biosteam® S Bag containing stoppers was pressurized with an Ammonia saturated gas (120Pa). A Bromo-Phenol cloth is placed over the surface for 5 minutes. A color change from yellow to blue, indicating cloth contact with Ammonia, indicates a leak.

## Results

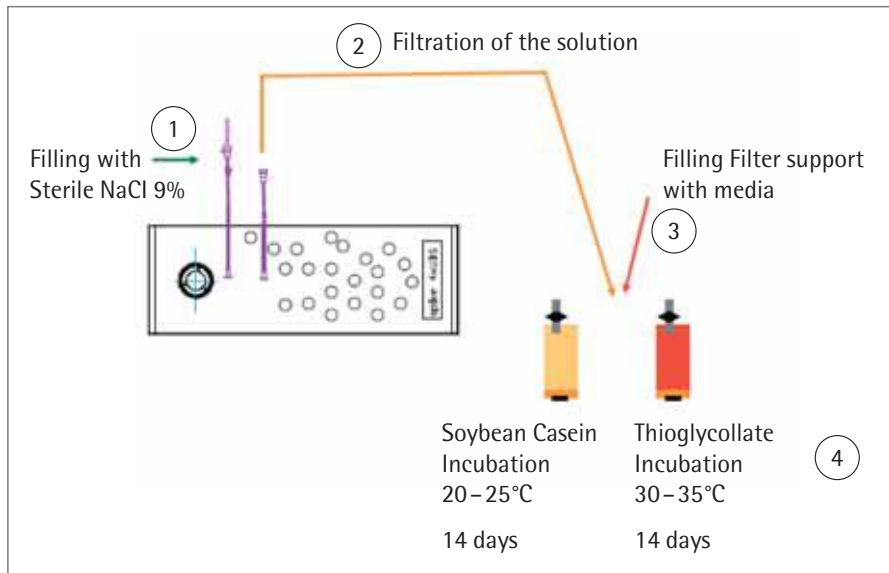
Tests	Acceptance criteria	Status
Integrity test on 35 Biosteam® S Bags filled with stoppers	No leak detected within 5 minutes	Pass (no leak)

## Sterility Test

**Objective:** Stoppers and packaging sterility testing after transfer

**Sampling:** 1 out of 3 Biosteam® S Bags filled with 1 kg of stoppers (a total of 32 units) were tested for sterility. Special Biosteam® S Bags were designed to avoid any contamination risk during sterility testing.

**Method (refer to diagram 5):** 1L of sterile sodium chloride solution was injected (1) inside the Biosteam® S Bag containing stoppers. Extracts (2) were then tested by applying the membrane filtrating test method (3, 4) according to the USP<71> [8] and E.P.2.6.1 [7] requirements.



**Diagram 5:** Sterility testing steps

## Results

Tests	Acceptance criteria	Status
Sterility test on 32 Biosteam® S Bags filled with stoppers	No microbial growth after 14-days incubation	Pass (no growth)

### Validation Results Analysis

The integrity tests and the sterility tests performed on sampling from the three sterilization cycles are conform to the expected results.

Mechanical and microbial qualification study of the stopper processing system (SPS) and the disposable Biosteam® S Bag assures the integrity of the whole system before, during and after fractional unloading (up to 40 connections and disconnections).

These tests performed on the disposable Biosteam® S Bag filled with stoppers prove the efficacy of the aseptic multiple transfers.

### Conclusion

The validation of the transfer of stoppers from a stopper processing system, using the Biosteam® S aseptic transfer technology, demonstrated the maintenance of sterility of the stoppers after their transfer into sterile single-use bags in an ISO Class 7 or 8 environment:

- Stopper steam sterilization (SAL 10<sup>-6</sup>) confirmed
- Empty Biosteam® S Bag Gamma sterilization (SAL 10<sup>-6</sup>) confirmed
- Biosteam® S Bag integrity confirmed
- Sterility test on bags with stoppers after transfer from the processing system conform (32 bags passed out of 32 tested) confirmed

These results demonstrate that the Biosafe® Biosteam® S technology offers enhanced sterility assurance in the stopper processing area, including both safety of stopper transfer and ease-of-use for in-house processing.

## **Bibliography**

- [1] PharmPro: "Pharmaceutically Clean, Validated, Elastomeric Components", November 2007
- [2] FDA Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Processes, 2004
- [3] NF EN ISO 14644-1: 1999 Clean rooms and associated controlled environments-Part 1 Classification of air cleanliness
- [4] 21 C.F.R. § 211.94 – Code of Federal Regulations Title 21- PART 211 Current Good Manufacturing Practice for Finished Pharmaceuticals - Subpart E - Control of Components and Drug Product Containers and Closures - § 211.94 Drug product containers and closures
- [5] PDA Technical Report No. 16, "Effects of Gamma Irradiation on Elastomeric Closures", 1992
- [6] NF EN ISO 11737-1: 2006 Sterilization of medical devices – Microbiological methods Part 1 - Determination of a population of micro-organisms on products
- [7] European Pharmacopeia 6th edition chapter 2.6.1 Sterility
- [8] US Pharmacopeia USP <71> Sterility Tests
- [9] PDA Technical Monograph No.1 : Industrial Moist Heat Sterilization In Autoclaves
- [10] NF EN ISO 11137: 2006 Sterilization of health care products – Radiation
- [11] ISO15747: Plastic containers for intravenous injections

## A Profile of Sartorius Stedim Biotech

Sartorius Stedim Biotech is a leading provider of cutting-edge equipment and services for the development, quality assurance and production processes of the biopharmaceutical industry. Its integrated solutions covering fermentation, filtration, purification, fluid management and lab technologies are supporting the biopharmaceutical industry around the world to develop and produce drugs safely, timely and economically. Sartorius Stedim Biotech focuses on single-use technologies and value-added services to meet the rapidly changing technology requirements of the industry it serves. Strongly rooted in the scientific community and closely allied with customers and technology partners, the company is dedicated to its philosophy of 'turning science into solutions'.

Headquartered in Aubagne, France, Sartorius Stedim Biotech is listed on the Euronext of Euronext Paris. With its own manufacturing and R&D sites in Europe, North America and Asia and a global network of sales companies, Sartorius Stedim Biotech enjoys a worldwide presence. Its key manufacturing and R&D site is in Germany.

Sartorius Stedim Biotech GmbH  
August-Spindler-Strasse 11  
37079 Goettingen, Germany

Phone +49.551.308.0  
Fax +49.551.308.3289  
[www.sartorius-stedim.com](http://www.sartorius-stedim.com)

USA Toll-Free +1.800.368.7178  
UK +44.1372.737159  
France +33.442.845600  
Italy +39.055.63.40.41  
Spain +34.90.2110935  
Japan +81.3.3740.5407

Specifications subject to change  
without notice. Printed and copyrighted  
by Sartorius Stedim Biotech GmbH  
W · G  
Publication No.: SBT4001-e11011  
Order No.: 85032-538-73  
Ver. 01 | 2011