ProConnex® Steam2® Valve Validation Report

Validation Report

Introduction

The Validation Report provides product information for Repligen ProConnex® Steam2® Valves. As an engineer, scientist, or manufacturer, you may need this information to guide your validation activities, including process development, writing validation protocols, and scaling up systems. Repligen is committed to providing all relevant technical, manufacturing, and quality information; however, only non-confidential information is presented in this document. Confidential details may be made available upon request through a formal confidentiality agreement or as part of a supplier audit.

Where to get help

If you need to know more about the ProConnex Steam2 Valves, contact the technical support team at Repligen. The technical support team includes scientists and engineers that can answer technical questions, assist in the selection of products, and provide user training.

Quality Documentation

Copies of the Repligen quality policy and ISO certificate can be found at repligen.com/resources.
To meet the needs of GMP manufacturing, these products are manufactured in the USA under the following quality standards: Repligen maintains an ISO 9001-compliant Quality Management System that is certified by BSI Group. A copy of the ISO certifications can be downloaded from repligen.com/resources.

- All materials in the direct fluid contact path meet USP Class VI and USP <88> requirements for in vivo Biological Reactivity.
- All fluid contact components are free from materials of animal origin or are compliant with EMA 410/01 Rev 3.

Objective

The ProConnex® Steam2® Valve, powered by innovative Artesyn® technology, is designed to make a sterile connection between a stainless steel bioreactor and single-use bags containing media.The valve consists of a stainless steel coupling and a silicone liner with a 1” flange connection. A series of tests were carried out to verify performance. Valves of two sizes (3/8” ID and 1” ID) along with accompanied liners were tested in parallel. Liners were gamma-irradiated before testing.
The study was divided into four sections:

- Mechanical Test
- Functional Test
- Bacterial Ingress Test
- Bacterial Sterilization Test

Test Descriptions

Mechanical Test: Both pressured steam cycles and air pressure were used to demonstrate seal integrity. The. The 1” ID valves and liners were subjected to three pressured steam cycles at a nominal temperature of 131°C ± 2°C for 75 minutes. Following pressured steam, the valves were exposed to 6 bar (87 psi) air pressure for 15 minutes. Valves were tested in both the valve closed and valve open configurations. Results show that all test units passed the leak test.

REPLIGEN

- **Functional Test:** The sterility assurance seal between a plug and the liner was tested with the use of an automated pressure tester. The pressure was incrementally increased by 50 mbar (0.73 psi) until a leak was observed. The liner with the 1" ID valve leaked at 350 mbar (5.1 psi). The liner with the 3/8" ID valve leaked at 1550 mbar (22.48 psi).
- **Bacterial Ingress Test:** Valves of two sizes were subjected to bacterial challenge. The test was carried out at three different stages during the valve installation. The plug seal, the pinch valve seal, and the liner flow path were tested by challenging with *B. diminuta* at a concentration of $>10^7$ CFU/mL. Results of the test show that all units tested negative for bacterial ingress after the seven-day incubation period.
- **Bacterial Sterilization Test:** Biological indicators containing spores of *G. stearothermophilus* were used to demonstrate that contaminants introduced to a flow path of tested valves can be effectively killed by steam sterilization at 121°C for 20 minutes. 1" ID and 3/8" ID valves were used in both horizontal and vertical configurations. Results show that all tested units were successfully sterilized in both configurations.

Table 1. Summary of the results for the 3/8" ID valves: LS – Small Liner (3/8"ID)

Test		Liner	Sterilization conditions	Pressure test pre sterilization	Pressure test post sterilization	Sterility result
Mechanical Test	Leak test	N/A				
	Hydraulic test					
Functional Test	Testing	LS51	N/A	1550 mbar	N/A	N/A
Bacterial Ingress Test	Plug challenge	LS4	N/A	N/A	N/A	No growth
		LS5	N/A	N/A	N/A	No growth
		LS6	N/A	N/A	N/A	No growth
	Closed valve	LS7	N/A	N/A	N/A	No growth
		LS8	N/A	N/A	N/A	No growth
		LS9	N/A	N/A	N/A	No growth
	Valve connection	LS10	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS11	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS12	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
Sterilization Testing	Horizontal	LS21	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS22	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS23	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
	Vertical	LS31	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS32	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LS33	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth

Table 2. Summary of the results for the 1" ID valves. LL – Large Liner (1”ID)

Test		Liner	Sterilization conditions	Pressure test pre sterilization	Pressure test post sterilization	Sterility result
Mechanical Test	Leak test	LL44	131°C ± 2°C x 75 min	N/A	Pass (no leaks)	N/A
		LL45	131°C ± 2°C x 75 min	N/A	Pass (no leaks)	N/A
		LL46	131°C ± 2°C x 75 min	N/A	Pass (no leaks)	N/A
	Hydraulic test	LL41	131°C ± 2°C x 75 min	N/A	N/A	N/A
		LL42	131°C ± 2°C x 75 min	N/A	N/A	N/A
		LL43	131°C ± 2°C x 75 min	N/A	N/A	N/A
Functional Test	Testing	LL51	N/A	350 mbar	N/A	N/A
Bacterial Ingress Test	Plug challenge	LL4	N/A	N/A	N/A	No growth
		LL5	N/A	N/A	N/A	No growth
		LL6	N/A	N/A	N/A	No growth
	Closed valve	LL7	N/A	N/A	N/A	No growth
		LL8	N/A	N/A	N/A	No growth
		LL9	N/A	N/A	N/A	No growth
	Valve connection	LL10	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL11	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL12	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
Sterilization Testing	Horizontal	LL211	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL211	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL211	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
	Vertical	LL31	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL32	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth
		LL33	121°C ± 2°C x 20 min	2 bar x 15 min/triplicate	N/A	No growth

[1]The same liner was used for each of three sterility tests, with a new bacterial strip inserted for each test.

Test summary

Mechanical Test

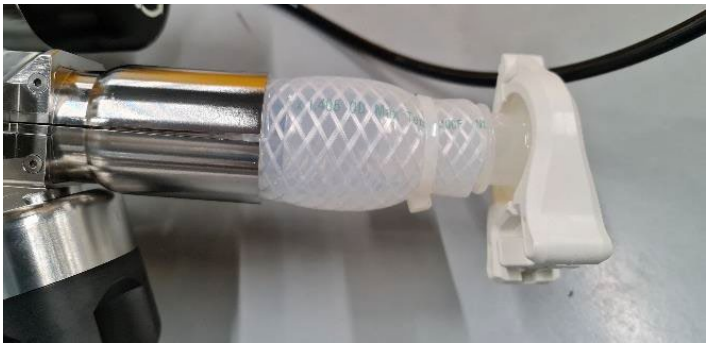
Both pressured steam and air pressure were used to demonstrate seal integrity of the large valves. The 1” ID valves and liners were subjected to three pressurized steam cycles at a nominal temperature of 131°C ± 2°C for 75 minutes. The valves were then exposed to 6 bar (87 psi) air pressure for 15 minutes. Valves were tested in both the valve closed (Figure 1) and valve open(Figure 2) configurations. Results (Table 2) show that all test units passed the leak test.

Table 3. Leak test requirements

Parameter	Requirement
Steaming method	Steam-in-place (SIP)
Number of steam cycles	3
Test process fluid	Air
Test pressure	6 bar for 15 minutes (flange side)
Acceptance criteria	No leaks
Valve position during test	Open/closed

Figure 1. Closed valve assembly for the pressure leak test

Figure 2. Open valve configuration with dead-ended tubing



Functional Testing

The goal of the test was to determine the pressure at which the TC barb plug stops sealing the unsupported liner. The test was performed on valves of both sizes. Each liner was connected to a pressure system (Figure 3) and pressurized at a 50 mbar (0.73 psi) until leakage. The opposite end of the liner was submerged underwater to observe a leak (Figure 4). For both valve sizes, the test was terminated after the plug was ejected from the liner. No air leak was observed before the plug ejection. The pressure at which the plug was released was recorded by the automated leak tester (Table 1, Table 2).

Table 4. Functional Testing requirements

Parameter	Requirement
Number of cycles	1
Test process fluid	Air
Test pressure	300 – 2500 mbar in 50 mbar increments
Acceptance criteria	No leaks

Figure 3. Valve assembly for Functional Testing

Figure 4. Plug submerged in water

Bacterial Ingress Testing

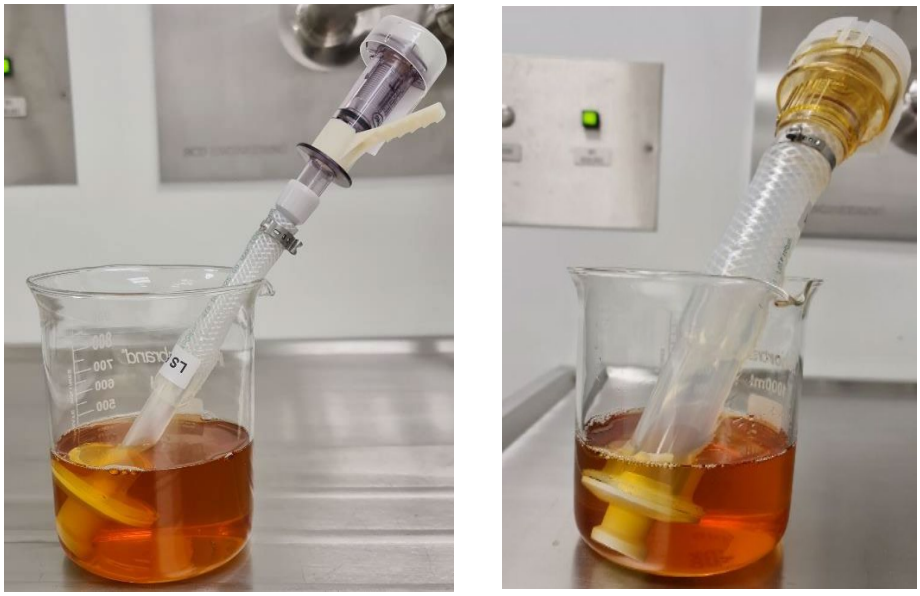
The purpose of the Bacterial Ingress Testing was to demonstrate that, after a SIP (steam-in-place) cycle, a sealed liner exposed to a non-sterile environment would maintain sterility. The test consisted of three phases: plug challenge, closed valve, valve connection. Each phase focused on sterility testing during normal liner installation. The testing was performed on valves of both sizes. The goal of the study was to verify that:

- The plug placed in the liner protects it from contaminants.
- The actuated valve prevents contaminants from getting into the sterile part of the liner.
- Contaminants introduced to the flange side of the liner can be effectively killed by 20-minute SIP cycle at 121°C ± 2°C.

Plug challenge

The flange-side of a liner was exposed to a B. diminuta suspension for five minutes (Figure 11). A bag containing tryptic soy broth (TSB) was connected to the other, non-exposed, side of the liner with a Pall Kleenpak™ sterile connector. The TSB was transferred from the bag into the liner, and held for five minutes to capture potential contaminants. At the conclusion of the exposure time, the contents of the liner were shifted back into the bag, which was disconnected aseptically using a Sartorius biosealer. The bag was incubated for seven days at 35°C to test for sterility. Results (Table 1, Table 2) show that the plug prevented contaminants from entering the sterile liner.

Figure 5. 3/8" ID (left) and 1: ID (right) submerged in *B. diminuta* suspension

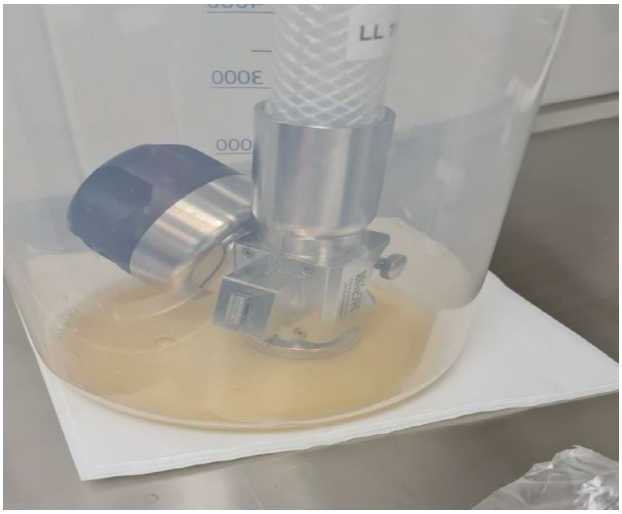


Closed valve

Each liner, with the plug attached to the flange side, was installed in the valve and the valve was closed. Once the valve was fully closed and the pin was flush with the handle, the plug was removed and the flange was exposed to *B. diminuta* suspension for a total time of 10 minutes (5 minutes upside-down (Figure 6) and 5 minutes submersed (Figure 7) to represent the worst-case scenario. After 10 minutes, a bag with tryptic soy broth (TSB) was connected, and a flush was performed. Results (Table 1, Table 2) show that the closed valve prevented contaminants from entering the sterile liner.

Figure 6. Valve in upside-down position. The cavity was filled with bacterial suspension for 5 minutes.

Figure 7. Valve dipped in bacterial suspension for 5 minutes



Valve connection test

Each liner was exposed to process air at 2 bar for 45 minutes total (3 x 15 minutes) and then to *B. diminuta* suspension. After exposure, the valve was connected to the vessel and steamed-in-place (Figure 8, Figure 9) for 20 min at 121°C ± 2°C. After sterilization, the two valves adjacent to the testing valve (Figure 10) were closed to provide a sterile boundary. A bag with TSB was connected and the valve was opened to expose the steamed part of the liner. A TSB flush was carried out for 10 minutes, after which the contents were transferred back to the bag, sealed, and incubated. Results show that no growth occurred following incubation (Table 1, Table 2).

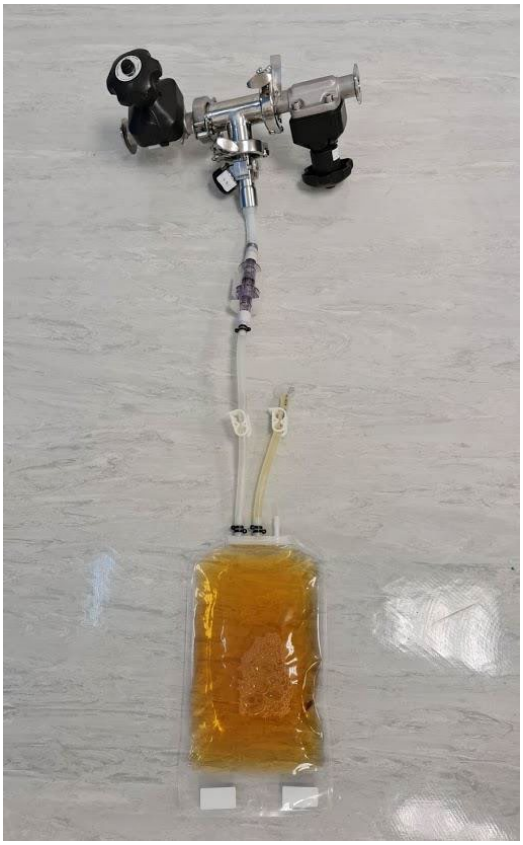
Table 5. Bacterial challenge valve connection test requirements

Parameter	Requirement
Steaming method	Steam-in-place
Steaming conditions	121°C ± 2°C for 20 minutes
Number of steam cycles	1
Bacterial organism	*B. diminuta*
Hold step before steaming	2 bar for 15 minutes three times
Acceptance criteria	No growth after incubation
Valve position during test	Open/closed

Figure 8. 3/8’ ID valve connected to steaming vessel

Figure 9. 1” ID valve connected to steaming station

Figure 10. Tested valve shown with adjacent hand valves and TSB in a single-use bag assembly with a Kleenpak connector



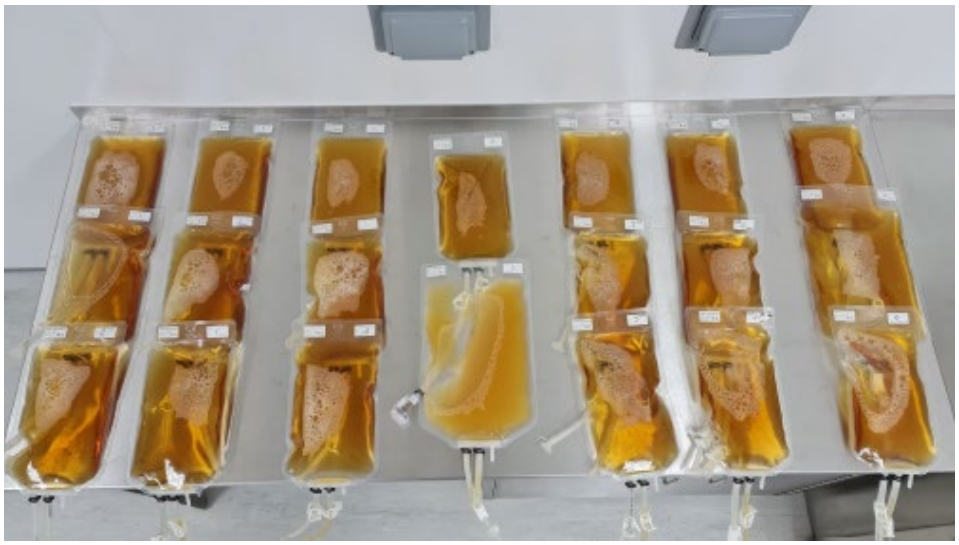
Bacterial Sterilization Testing

The goal of this test is to verify that the valve is sterile after a steam cycle at 121°C ± 2°C for 20 minutes. Each liner was secured into the valve and the plug was removed. The assembled valve was exposed to process air at 2 bar (29 psi) for 45 minutes total (3 x 15 minutes). A strip with G. stearothermophilus was then placed inside the liner at the flange side. The valve was connected to the steaming station and the sterilization was carried out for 20 min at 121°C ± 2°C. Following sterilization, each strip was removed from the valve, transported into a biological safety cabinet, and aseptically transferred into a vial with TSB. Results show no growth for both valve sizes in both the vertical and horizontal positions (Table 1, Table 2).

Table 6. Bacterial Sterilization Testing requirements

Parameter	Requirement
Steaming method	Steam-in-place
Steaming conditions	121°C ± 2°C for 20 minutes
Number of steam cycles	1
Bacterial organism	*G. stearothermophilus (microstrips)*
Hold step before steaming	2 bar for 15 minutes three times (flange side)
Acceptance criteria	No growth after incubation
Valve position during test	Closed
Valve position on system	Vertical/horizontal

Figure 11 Bacterial challenge results



Legend

LL 4	LL 7	LL 10	Negative Control	LS 4	LS 7	LS 10
LL 5	LL 8	LL 11	Positive Control	LS 5	LS 8	LS 11
LL 6	LL 9	LL 12		LS 6	LS 9	LS 12

Conclusion

This report demonstrates that the Repligen ProConnex Steam2 Valve passed all functional and bacterial challenge tests outlined in this study. This testing represents worst case scenarios to guide a customer's validation activities, including process development, writing validation protocols, and scaling up systems.

Part numbers

Table 7. Valve configurations

Part Number	Inlet Type	ID (with SU Installed)	Method 0f Operation
S2SAE0375X062516	1" – 1 1/2" TC	0.375"	Manual
S2SAE0500X075016	1" – 1 1/2" TC	0.500"	Manual
S2SAE0750X112516	1" – 1 1/2" TC	0.750"	Manual
S2SAE1000X140516	1" – 1 1/2" TC	1.00"	Manual

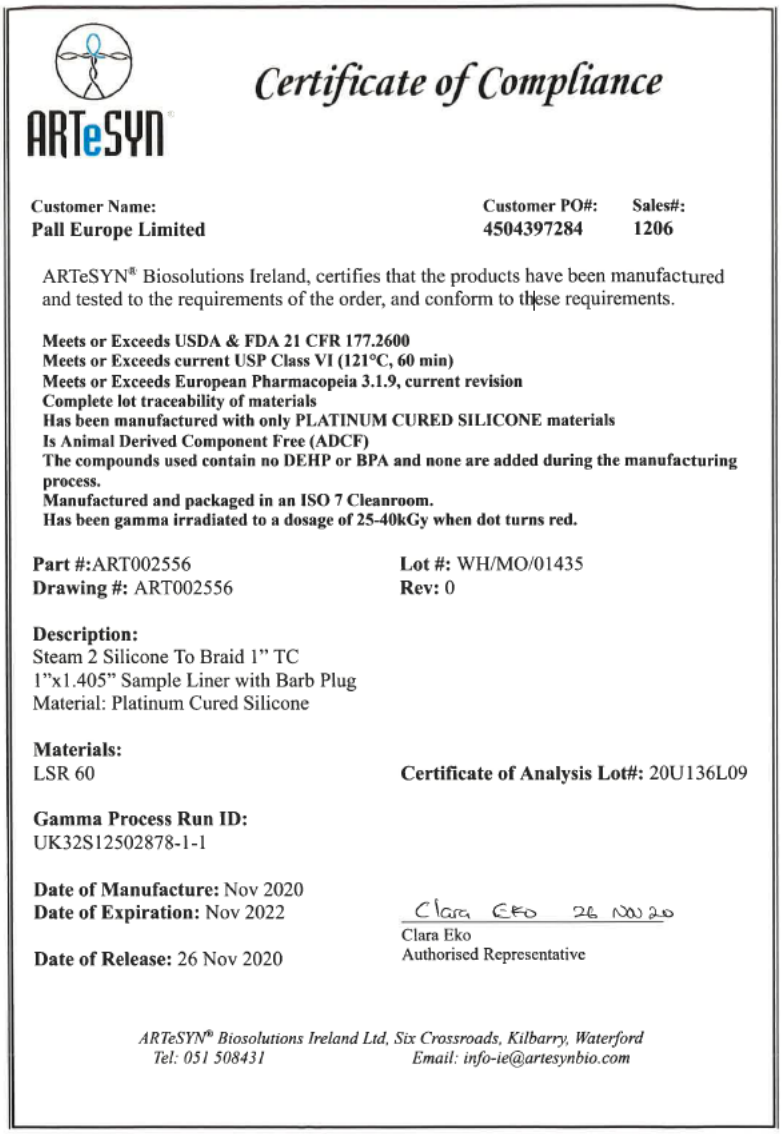
Table 8. Consumables configurations

Part Number	Inlet Type	ID (with SU Installed)	Braid/Clear Silicone	Outlet [CPC]
S2SBAE0375X0625AQG	1" – 1 1/2" TC	0.375"	Braid	AQG17006
S2SBAE0500X0875AQG	1" – 1 1/2" TC	0.500"	Braid	AQG17008
S2SBAE0750X1125AQG	1" – 1 1/2" TC	0.750"	Braid	AQG17012
S2SBAE1000X1405AQG	1" – 1 1/2" TC	1.00"	Braid	AQL17016
S2SSAE0375X0625AQG	1" – 1 1/2" TC	0.375"	Clear	AQG17006
S2SSAE0500X0875AQG	1" – 1 1/2" TC	0.500"	Clear	AQG17008
S2SSAE0750X1125AQG	1" – 1 1/2" TC	0.750"	Clear	AQG17012
S2SSAE1000X1405AQG	1" – 1 1/2" TC	1.00"	Clear	AQL17016

Note: *Other customized solutions and sizes to suit specific applications are available upon request. In cases where the design of the closure position distance from the connection is increased/altered to accommodate application parameters this validation should only be used as reference.*

Appendix A: Certificate of Compliance

Figure 12. Certificate of Compliance



ARTeSYN

Certificate of Compliance

Customer Name: **Pall Europe Limited**

Customer PO#: **4504397284**

Sales#: **1206**

ARTeSYN® Biosolutions Ireland, certifies that the products have been manufactured and tested to the requirements of the order, and conform to these requirements.

Meets or Exceeds USDA & FDA 21 CFR 177.2600
Meets or Exceeds current USP Class VI (121°C, 60 min)
Meets or Exceeds European Pharmacopeia 3.1.9, current revision
Complete lot traceability of materials
Has been manufactured with only PLATINUM CURED SILICONE materials
Is Animal Derived Component Free (ADCF)
The compounds used contain no DEHP or BPA and none are added during the manufacturing process.
Manufactured and packaged in an ISO 7 Cleanroom.
Has been gamma irradiated to a dosage of 25-40kGy when dot turns red.

Part #:ART002556
Drawing #: ART002556

Lot #: WH/MO/01435
Rev: 0

Description:
Steam 2 Silicone To Braid 1" TC
1"x1.405" Sample Liner with Barb Plug
Material: Platinum Cured Silicone

Materials:
LSR 60

Certificate of Analysis Lot#: 20U136L09

Gamma Process Run ID:
UK32S12502878-1-1

Date of Manufacture: Nov 2020
Date of Expiration: Nov 2022

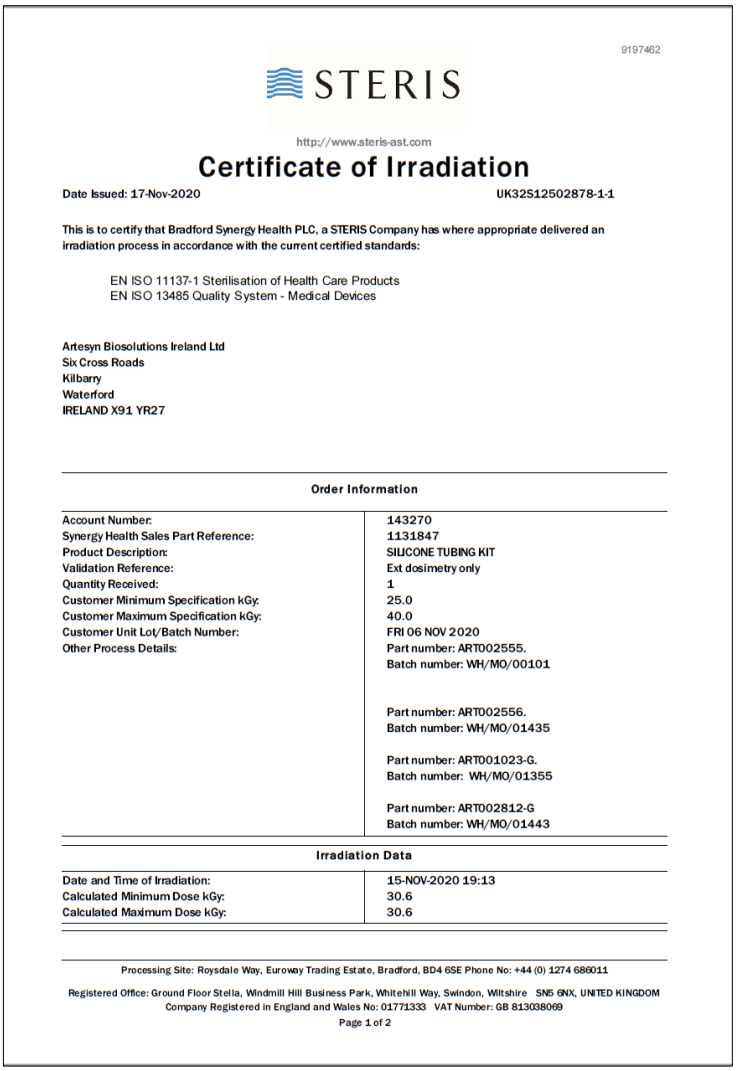
Date of Release: 26 Nov 2020

Clara Eko 26 Nov 20
Clara Eko
Authorised Representative

ARTeSYN® Biosolutions Ireland Ltd, Six Crossroads, Kilbarry, Waterford
Tel: 051 508431 *Email: info-ie@artesynbio.com*

Appendix B: Certificate of Irradiation

Figure 13. Certificate of Irradiation, page 1 of 2



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STERIS

http://www.steris-ast.com

Certificate of Irradiation

Date Issued: 17-Nov-2020 UK32S12502878-1-1

This is to certify that Bradford Synergy Health PLC, a STERIS Company has where appropriate delivered an irradiation process in accordance with the current certified standards:

EN ISO 11137-1 Sterilisation of Health Care Products
EN ISO 13485 Quality System - Medical Devices

Artesyn Biosolutions Ireland Ltd
Six Cross Roads
Kilbarry
Waterford
IRELAND X91 YR27

Order Information

Account Number:	143270
Synergy Health Sales Part Reference:	1131847
Product Description:	SILICONE TUBING KIT
Validation Reference:	Ext dosimetry only
Quantity Received:	1
Customer Minimum Specification kGy:	25.0
Customer Maximum Specification kGy:	40.0
Customer Unit Lot/Batch Number:	FRI 06 NOV 2020
Other Process Details:	Part number: ART002555. Batch number: WH/MO/00101 Part number: ART002556. Batch number: WH/MO/01435 Part number: ART001023-G. Batch number: WH/MO/01355 Part number: ART002812-G Batch number: WH/MO/01443

Irradiation Data

Date and Time of Irradiation:	15-NOV-2020 19:13
Calculated Minimum Dose kGy:	30.6
Calculated Maximum Dose kGy:	30.6

Processing Site: Roysdale Way, Euroway Trading Estate, Bradford, BD4 6SE Phone No: +44 (0) 1274 686011

Registered Office: Ground Floor Stella, Windmill Hill Business Park, Whitehill Way, Swindon, Wiltshire SN5 6NX, UNITED KINGDOM
Company Registered in England and Wales No: 01771333 VAT Number: GB 813038069

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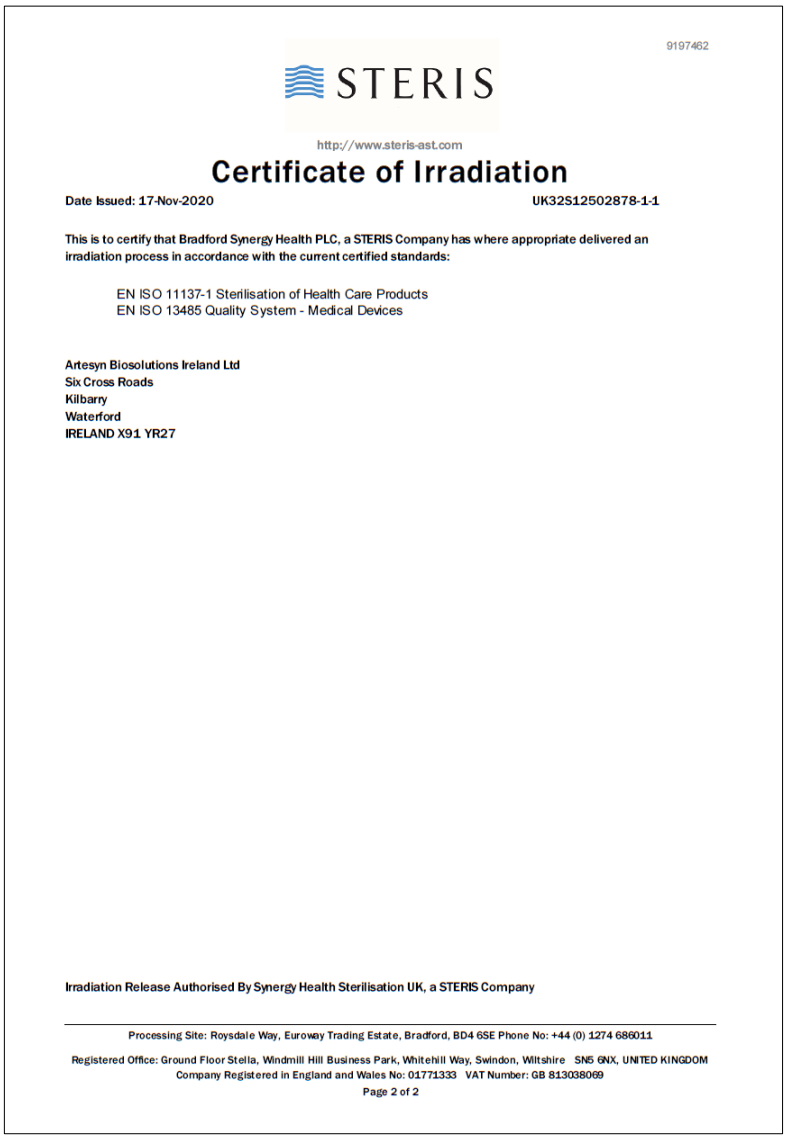
Figure 14. Certificate of Irradiation, page 2 of 2



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STERIS

http://www.steris-ast.com

Certificate of Irradiation

Date Issued: 17-Nov-2020 UK32512502878-1-1

This is to certify that Bradford Synergy Health PLC, a STERIS Company has where appropriate delivered an irradiation process in accordance with the current certified standards:

EN ISO 11137-1 Sterilisation of Health Care Products
EN ISO 13485 Quality System - Medical Devices

Artesyn Biosolutions Ireland Ltd
Six Cross Roads
Kilbarry
Waterford
IRELAND X91 YR27

Irradiation Release Authorised By Synergy Health Sterilisation UK, a STERIS Company

Processing Site: Roysdale Way, Euroway Trading Estate, Bradford, BD4 6SE Phone No: +44 (0) 1274 686011

Registered Office: Ground Floor Stella, Windmill Hill Business Park, Whitehill Way, Swindon, Wiltshire SN5 6NX, UNITED KINGDOM
Company Registered in England and Wales No: 01771333 VAT Number: GB 813038069

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