plasmapp

PLA-TIR-I-2021-M002-00

TEST·INSPECTION REPORT

Lumen Sterilization Test

Manufacturer Name: Plasmapp Co., Ltd.

Representative: Youbong, LIM

Location: BVC-111, 125, Gwahak-ro, Yuseong-gu, Daejeon, 34141, Republic of Korea

Product Name: Low temperature plasma sterilizer and sterilant

Brand Name: STERLINK™ and STERMATE™

Model Name: STERLINK mini and STERLOAD™ mini

Serial Number: M072BWH003A and SM20K013

Test·Inspection Item: Lumen Sterilization Test

Testing Laboratory: Plasmapp Research Institute

Location: 372, Dongbu-daero, Osan-si, Gyeonggi-do, 18151, Republic of Korea

Decision: Pass

Tested until: 29 Nov. 2021 **Issued Date:** 30 Nov. 2021

Test manager: 전 현 정
Hyun Jeong, JEON
Senior researcher

Approver:
Youbong, LIM
Chief Executive Officer

Plasmapp Research Institute

Lumen Sterilization Test

1. Test schedule

1.1 Date of test beginning: 4 Nov. 2021

1.2 Date of test completion: 29 Nov. 2021

2. Test article

Low temperature plasma sterilizer (STERLIN mini, S/N: M072BWH003A)

Sterilant (STERLOAD™ mini, Lot No.: SM20K013)

3. Test guideline

3.1 The tests were performed accordance with the standards of ISO 14937:2009, ISO 11737-1:2018 and ISO 11737-2: 2009.

3.2 Information of testing materials

3.2.1 Lumen*

Lumen type	Inner diameter [mm]	Length [mm]
Single-channel stainless steel lumen (Both side open)	1.6	200

3.2.2 Validation load*

Test sample	Validation load	
	Medical devices	Total weight [lbs]
Five lumens	Stainless steel scissors	1.54

3.2.3 Bacteria spore suspension

Item	Details
Product	Spores suspension
Model	BT20S/7
Manufacturer	Terragene S.A.
Lot Number	S20C112022
D-value	9 sec (50°C, 2 mg·L^{-1} VH_2O_2)
Survival time / Kill time	39 sec / 95 sec
Expiration date	Nov. 2022
Comments	1.8×10^6 *Geobacillus stearothermophilus* spores (ATCC Cell Line 7953) / 0.1 ml complying with ISO 11138

*Figures of the lumen and validation load were referred to the Appendix 1.

3.2.4 Sterilant

Brand	STERMATE™
Model	STERLOAD™ mini
Lot Number	SM20K013
Expiration date	Nov. 2021
Manufacturer	Plasmapp Co., Ltd.
Comments	Sterilant cassette for STERLINK mini

3.2.5 Measuring instrument

Equipment	Manufacturer	Model	Internal S/N	Calibration date
High temperature data logger	MADGETECH	HiTemp140	PO-C-025	16 Feb. 2021
Pressure data logger	MADGETECH	PR140	PQ-C-031	10 Feb. 2021

3.3 Test methods

3.3.1 Lumen leakage validation

(1) One side of lumen was directly connected to the test chamber which is connected to a vacuum pump (VP) via solenoid valve (SV) as depicted in Figure 2.1. Another side of lumen was blocked.

(2) The pressure data logger (PG) was located inside the test chamber.

(3) After sufficient pumping, the solenoid valve was closed in order to isolate the lumen and chamber system.

(4) If the lumen is leak-tight, the pressure raising rate after closing the valve is almost zero.

(5) The time evolutions of the pressure were recorded. (The criterion is less than 0.1 $Torr \cdot s^{-1}$.)

(6) Same tests without blocking one side of the lumen were conducted to clarify that there is no blockage in the lumen channel as described in Figure 2.2.

(7) All the data were recorded and plotted in Figure 3.1 and Figure 3.2.

3.3.2 Elution and recovery techniques

(1) Three lumens were prepared.

(2) The *Geobacillus stearothermophilus* spore suspension of 0.1 ml was inoculated on inside of each lumen, and dried.

(3) The inoculated area of the lumen was rubbed by a swab sterilized and moistened with tryptic soy broth. This process was repeated using a new swab twice for each.

(4) The swab heads were cut by a flame-sterilized scissor and placed in the tryptic soy broth of 5 ml.

(5) The tubes were mixed using a vortex mixer for 3 minutes to recover the spores from the swabs.

(6) The media containing recovered spores were diluted by serial 10-fold dilution method and they were inoculated in the tryptic soy agar as triplicate plates for each dilution.

(7) The inoculated plates were incubated at 60°C for 48 hours.

(8) After incubation, the number of recovered spores was determined by counting the number of colonies grown on tryptic soy agar.

(9) The recovered spores from each lumen should be greater than 1.0×10^6 colony forming unit (CFU).

3.3.3 Lumen sterilization test

(1) The *G. stearothermophilus* spore suspension of 0.1 ml was inoculated on inside of each lumen, and dried.

(2) Five lumens and validation load were inserted in Tyvek® and sealed. **For the worst-case condition, the sterilant which has a short expiration date was used.**

(3) The prepared pouch was processed with half cycle sterilization of chamber mode. The temperature and pressure during the sterilization process were measured.

(4) One inoculated lumen was left on the room temperature without sterilization process as positive control samples.

(5) After sterilization cycle, the inoculated area on all the lumens were rubbed by swab sterilized and moistened with tryptic soy broth. This process was repeated using a new swab twice for each.

(6) The swab heads were cut by a flame-sterilized scissor and placed in the tryptic soy broth of 5 ml.

(7) All samples were incubated at 60°C for 7 days. After incubation, all samples were mixed by a vortex mixer and the turbidity change of media was inspected. The results were reported as positive (growth) or negative (no growth).

(8) The lumen sterilization tests except the positive control test were performed in three consecutive half sterilization cycles.

4. Test results**

4.1. Results of lumen leakage validation

Test number	Pressure raising rate [Torr·s^{-1}] Criterion: < 0.1 Torr·s^{-1}				
	Lumen #1	Lumen #2	Lumen #3	Lumen #4	Lumen #5
1	0.076	0.012	0.013	0.066	0.097
2	0.032	0.007	0.007	0.002	0.027
3	0.006	0.007	0.019	0.072	0.065
w/o rubber	Open	Open	Open	Open	Open

4.2. The number of recovered spores from the lumen

Number of recovered spores [× 10^6 CFU]		
#1	#2	#3
1.52 ± 0.02	1.44 ± 0.03	1.46 ± 0.06

**The related figures were referred to the Appendix 3, 4, and 5. The time evolution of pressure and temperature inside the chamber during lumen sterilization test were described in the Appendix 6, as well.

4.3. Results of lumen sterilization test

Test type	Test number	Number of positive/Number of tested
Positive control		1/1
Three consecutive tests	1	0/5
	2	0/5
	3	0/5

4.4. Pressure parameter data during sterilization process [Torr]

Test number	Sterilization phase 1		Sterilization phase 2		Purification
	Base[a] (< 3 Torr)	Diffusion[b] (20 - 100 Torr)	Base[a] (< 3 Torr)	Diffusion[b] (20 - 100 Torr)	Final base[c] (< 3 Torr)
1	0.08	42.7	-	-	1.40
2	0.58	49.7	-	-	1.29
3	0.78	46.2	-	-	1.60

4.5. Temperature parameter data during sterilization process [°C]

Test number	Sterilization phase 1			Sterilization phase 2		
	Load[d] (40-60°C)	Chamber[e] (55-60°C)	Vaporizer[f] (110-130°C)	Load[d] (40-60°C)	Chamber[e] (55-60°C)	Vaporizer[f] (110-130°C)
1	48.3 – 58.4	57.9 – 58.2	113 – 122	-	-	-
2	48.2 – 59.2	58.2 – 58.8	117 – 124	-	-	-
3	47.6 – 59.3	57.9 – 58.3	112 – 121	-	-	-

4.6. Time parameter data during sterilization process [s]

Test number	Sterilization phase 1 (300 ± 1 s)	Sterilization phase 2 (300 ± 1 s)
1	300	-
2	300	-
3	300	-

[a]The base pressure just before injection of the sterilant.
[b]The diffusion pressure after diffusion of the sterilant which is complete.
[c]The base pressure after injection and purification.
[d]The load temperature is measured by the temperature data logger described in 3.2.5.
[e]The chamber temperature is controlled by K-type thermocouple.
[f]The vaporizer temperature is controlled by K-type thermocouple.

5. Conclusions

(1) The results of lumen leakage validation were confirmed that the lumens using in this test do not have the leakage.

(2) The recovered spores from each lumen were recovered as above 1.0×10^6 CFU.

(3) The results of three consecutive lumen sterilization tests were shown as all negative except positive control.

(4) According to the test results, the lumen sterilization test is completely successful.

Appendix 1

1. Shape of the lumen

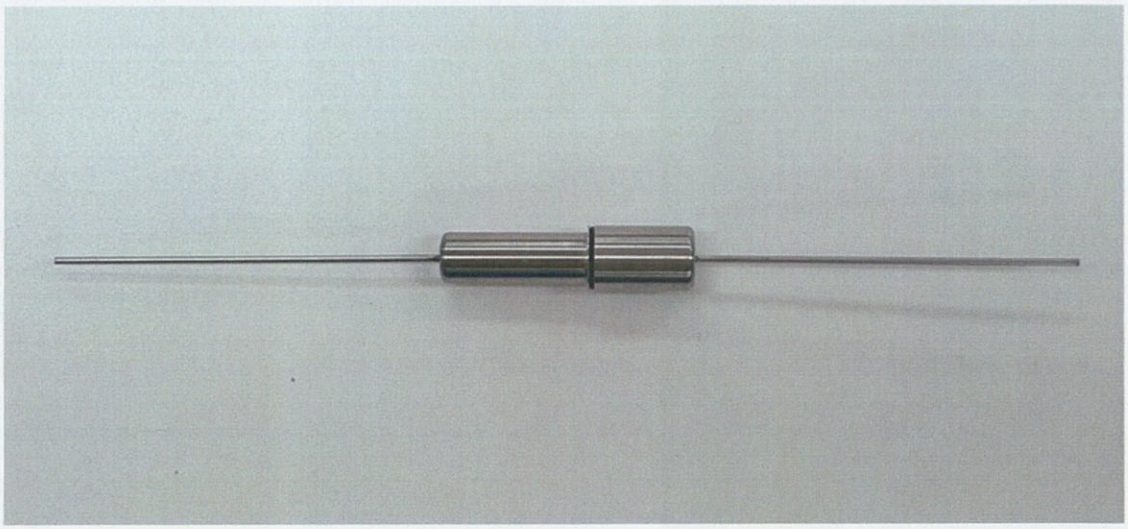

Figure 1.1 The shape of the lumen.

Figure 1.2 The inoculated area in the lumen is marked with a red diagonal line.

2. Chamber mode test

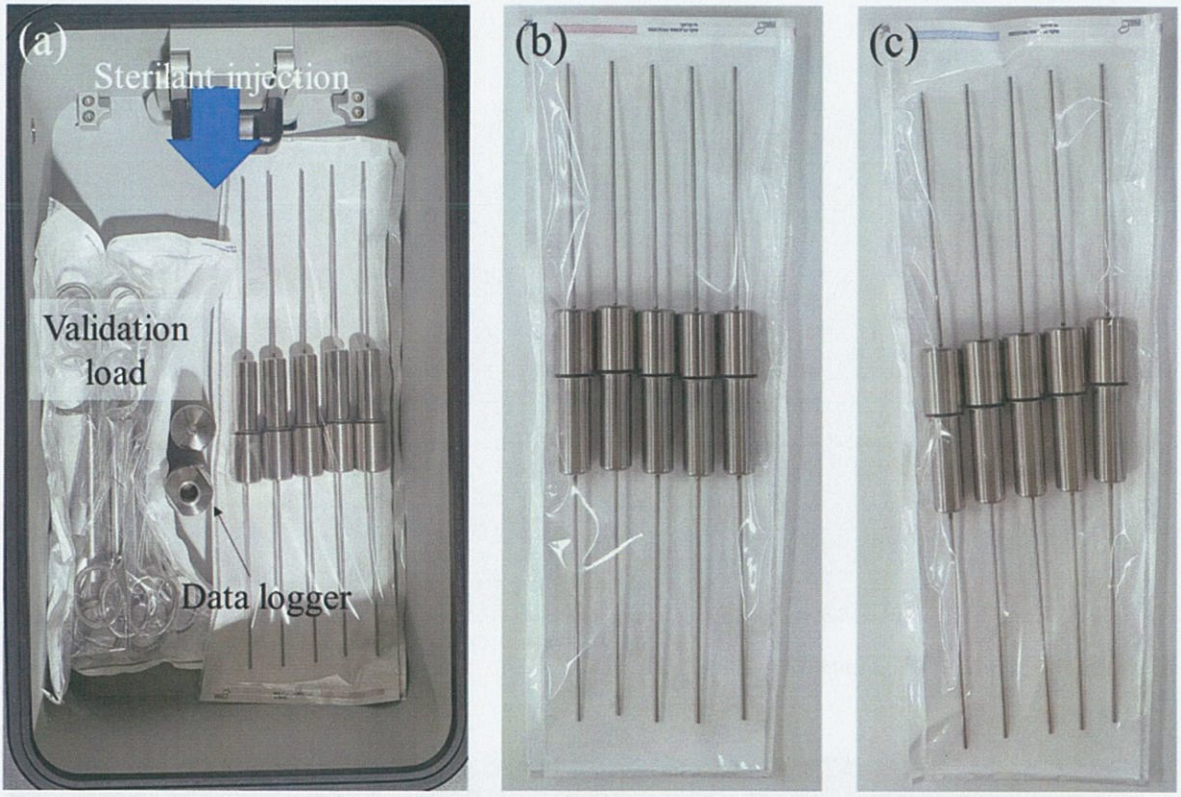

Figure 1.2 (a) The position of validation load and inoculated lumens sealed by Tyvek® pouch. The lumens are described in (b) before and (c) after the sterilization.

Appendix 2

1. Lumen leak-tight validation

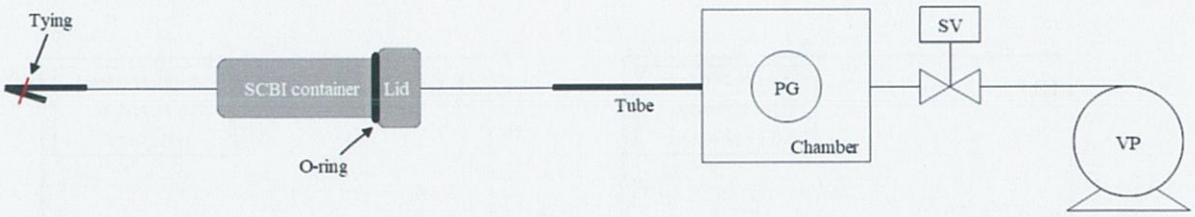

Figure 2.1 Apparatus of lumen leak-tight test for the lumen

2. Lumen open test

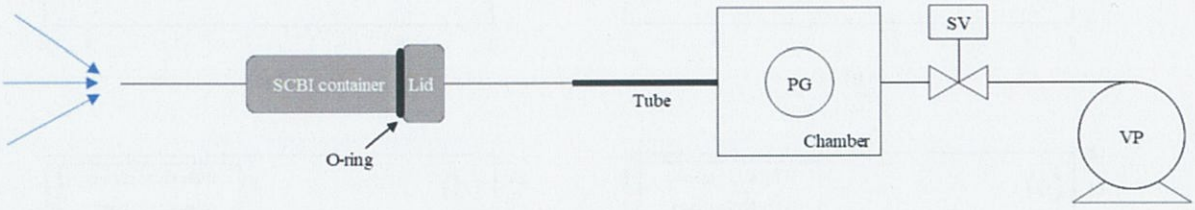

Figure 2.2 Apparatus of lumen open test without blocking

Appendix 3

1. Result of lumen leak-tight validation

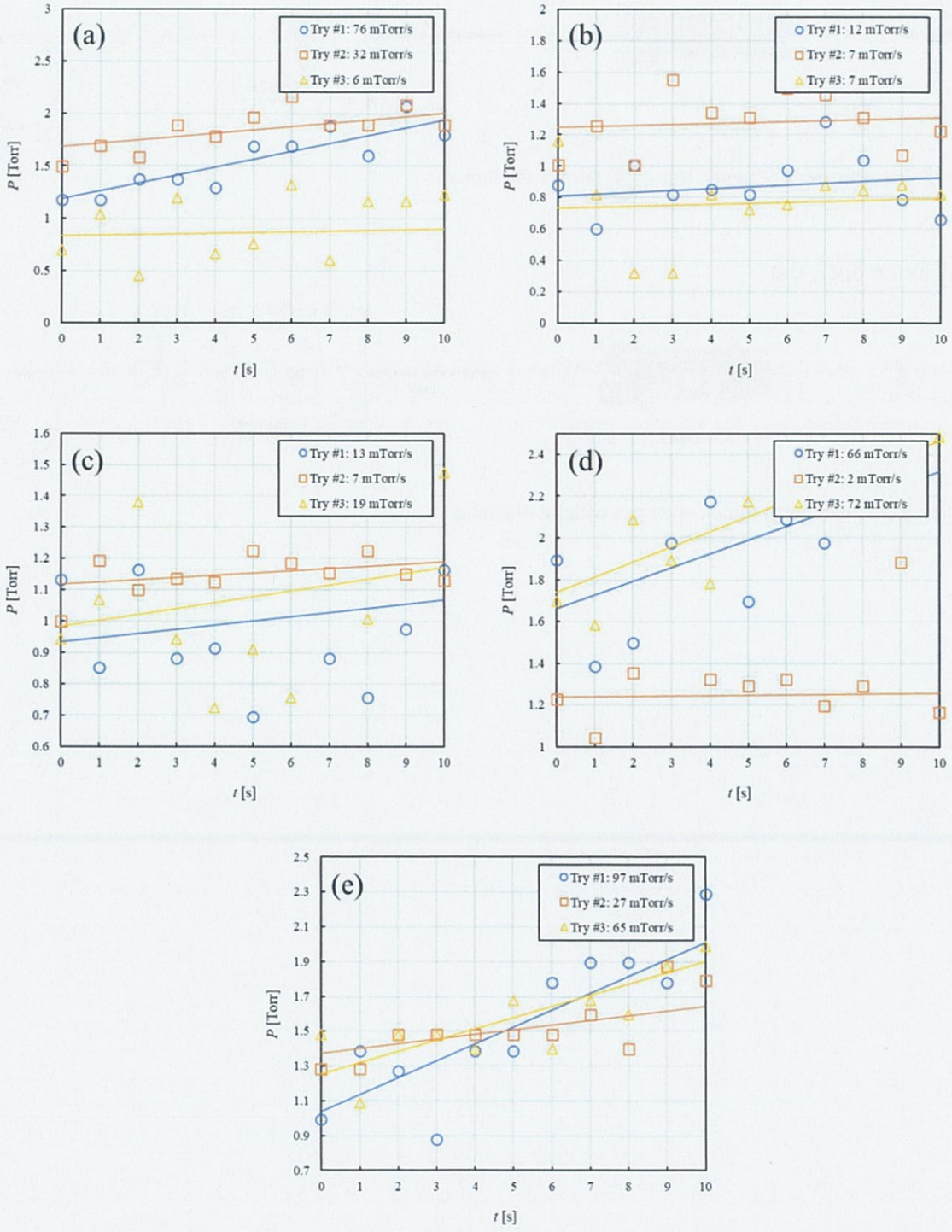

Figure 3.1 Pressure rising after closing the solenoid valve (SV) for (a) Lumen #1, (b) Lumen #2, (c) Lumen #3, (d) Lumen #4, and (e) Lumen #5.

2. Result of lumen open test

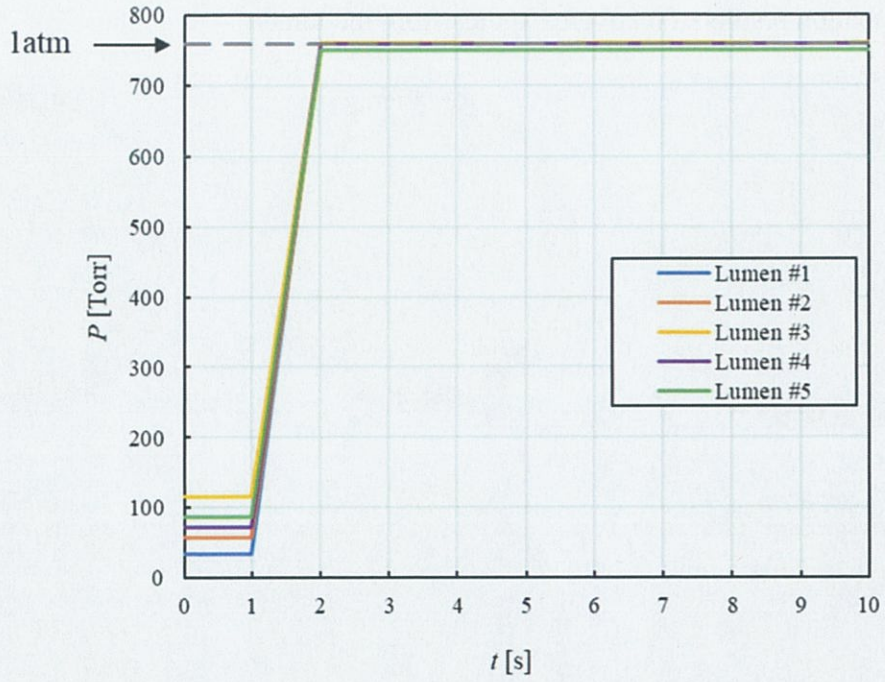

Figure 3.2 After the solenoid valve (SV) closed, the pressure reached atmospheric pressure. Accordingly, it is clarified that the lumen is properly connected to the vacuum pump.

Appendix 4

1. The incubation results of recovered spores from the lumen

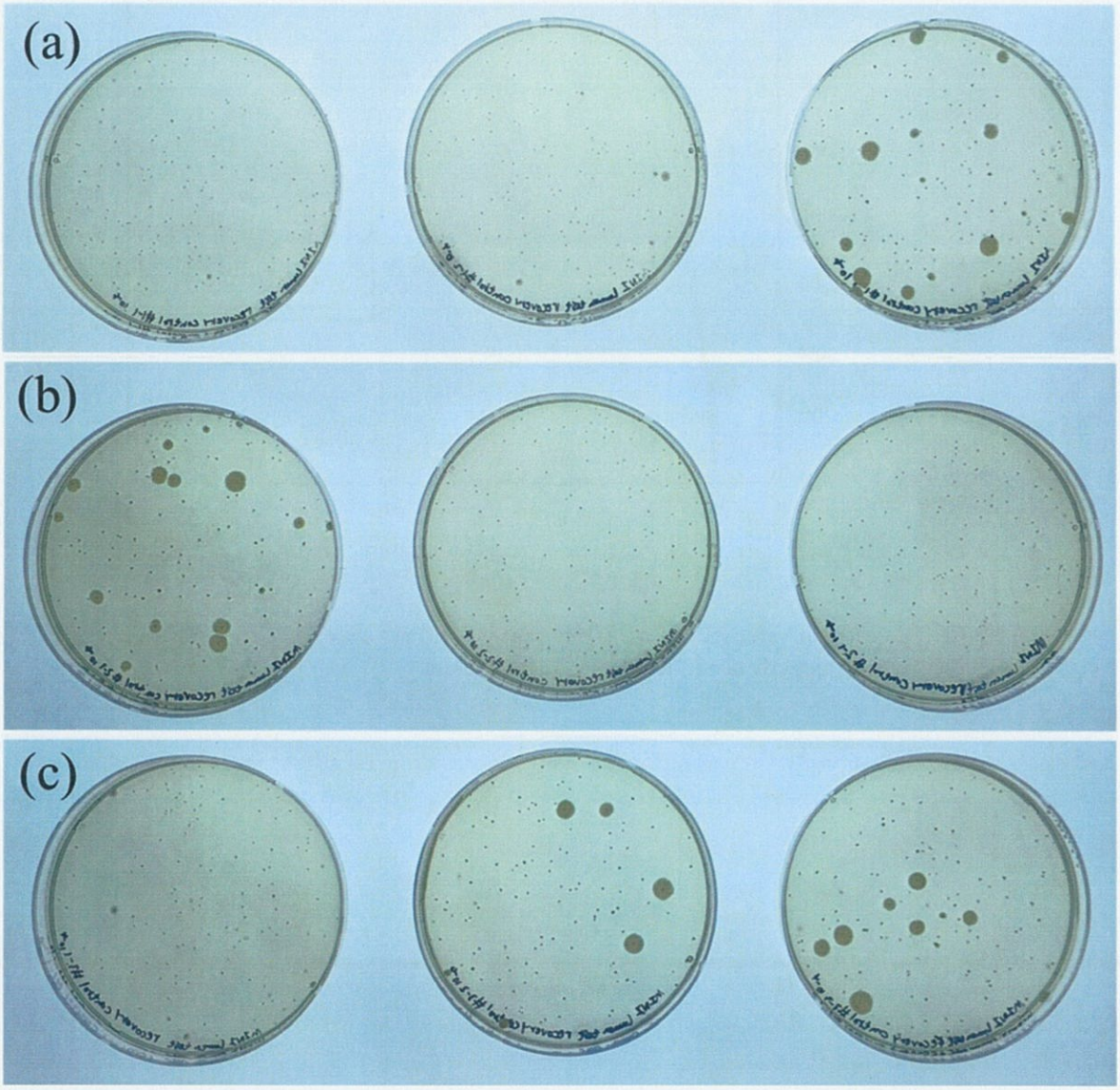

Figure 4.1 The incubation results of 10^{-4} dilution samples of recovered from the lumen.

Appendix 5

1. Results of the lumen sterilization test

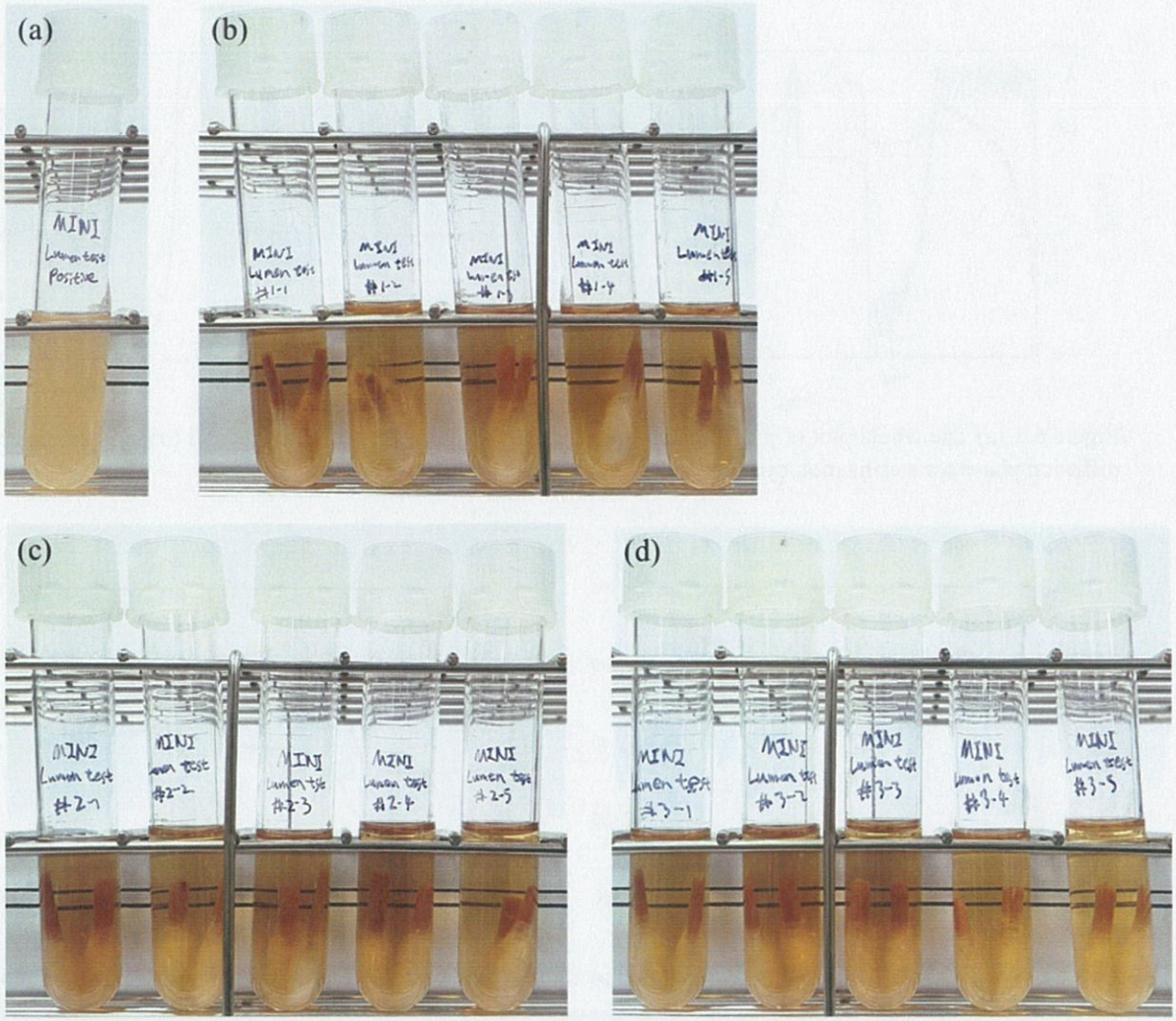

Figure 5.1 The incubation results of (a) the positive control and (b-d) three consecutive half sterilization cycles. The turbidity can be determined by black lines behind the tubes. All the test samples after the sterilization are transparent except the positive control.

Appendix 6

1. Pressure and temperature curves of the lumen sterilization test

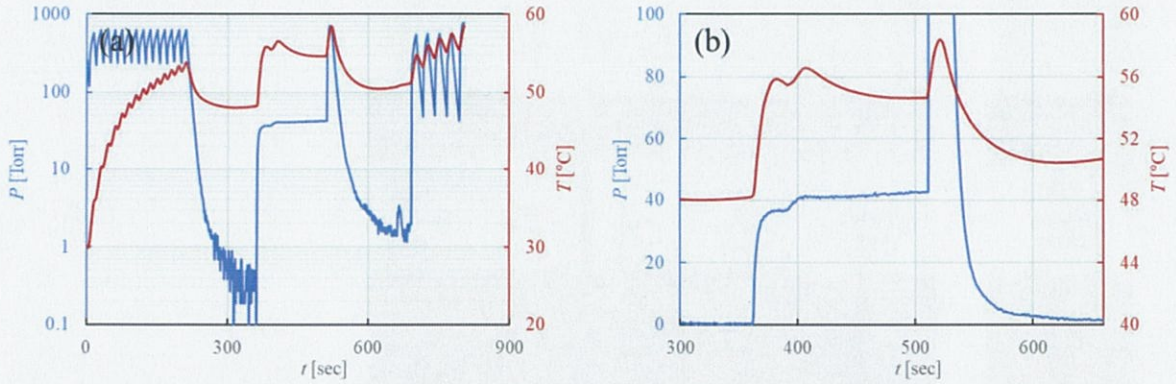

Figure 6.1 (a) The whole plot of pressure and temperature curve during the full cycle and (b) magnified plot of diffusion phase for sterilization cycle 1.

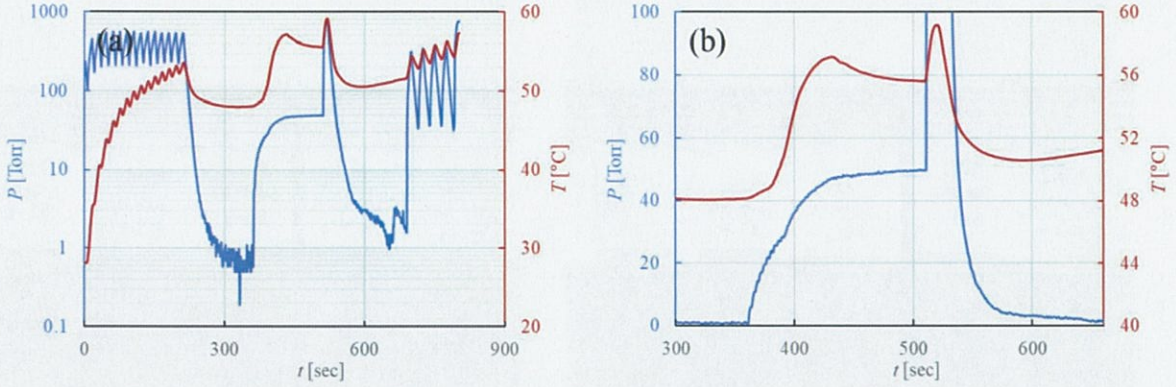

Figure 6.2 (a) The whole plot of pressure and temperature curve during the full cycle and (b) magnified plot of diffusion phase for sterilization cycle 2.

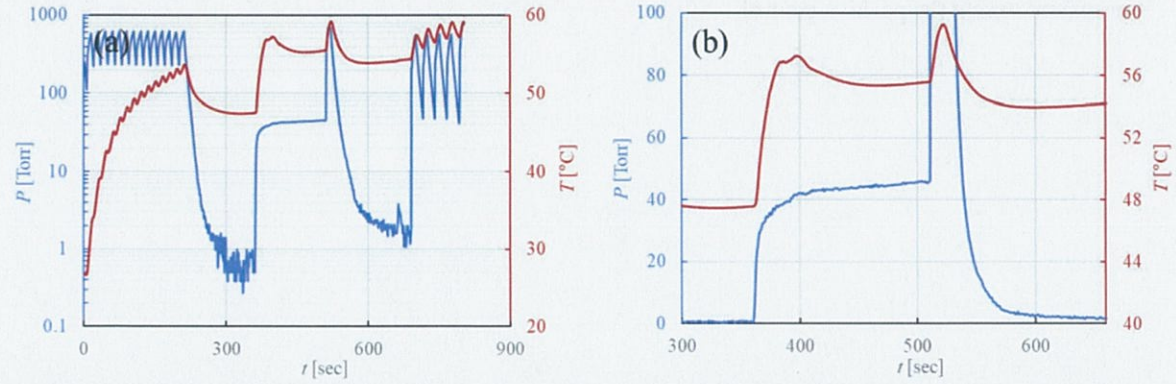

Figure 6.3 (a) The whole plot of pressure and temperature curve during the full cycle and (b) magnified plot of diffusion phase for sterilization cycle 3.