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Infected Carcass and Tissue Decontamination

This study was performed to evaluate and establish the parameters and requirements necessary for complete decontamination of infected murine carcasses using a Safe-Decon Model 200 Oval Container and a standard laboratory autoclave, Tuttnauer Model 69137 single door autoclave with load probes. The current process requires that the contaminated murine carcasses be temporarily placed into a plastic sealable bags and stored in a refrigerator/freezer until all the carcasses from a specific experiment can be sterilized *in toto*.

During standard decontamination procedures when the carcasses are processed for removal from the containment

laboratory the mice are typically in sealed ziplock bags and are then placed into an autoclavable bag (some facilities will double bag) within a Biological Safety Cabinet (BSC). The autoclave bag is sealed using autoclave tape then placed into a stainless-steel autoclave pan covered by a stainless steel lid or aluminum foil. The pan is then surfaced deconned and then transferred out of the BSC to the autoclave. If the bags are not opened there will be no exchange of steam/heat to the carcasses or contents, thus requiring additional time to heat the air that is trapped within the plastic bags in order to achieve sterilization temperature. According to the approved carcass sterilization protocols at multiple BSL labs we found that the cycling times to achieve complete sterilization ranged from 3 hours to as much as 7 hours.

In order to monitor in real-time the temperature and pressure we utilized autoclave load probes and discovered when you have a large carcass (or mass) in a sealed autoclavable bag, the lack of the steam/heat exchange into the bag results in a slow rise in temperature to the required 121°C/250°F. On average it required 180 minutes in the sterilization phase. What we also observed was that during the heat-up and sterilization phases that the internal temperatures were fluctuating as much as 20°C then the temperature would drop quickly and slowly recover back to 121°C/250°F. The recovery time observed was on average of 15 minutes. We believe that there are pockets of liquid within the tissues and, under pressure during the autoclave cycle, the tissue was being insulated from the heat by these liquids. We therefore decided to start running PreVac

cycles which would force out the liquids from the tissue, thus separating the solids from the liquids during the process using the Safe-Decon sealable/airtight Containers. The results demonstrated that the temperatures in the tissues were constant and consistent in achieving the required sterilization temperature. We were able to run total cycle times of 56-minutes (30-minute cycle with 2 lbs 8 oz of tissue) and achieved sterilization of both the tissue and the liquid materials.

Validation Procedure

Equipment

Autoclave: Tuttnauer, Model 69137 single door autoclave with load probes

Decon Container: Safe-Decon Inc. Model 200 Oval Container

Biological Indicator (BI): *Geobacillus sterothermophilus* autoclave biological indicators (BIs) [EZ Test MesaLabs Lot: S-534, Exp.2023-05-14].

BI Cultures: BIs were incubated at 60°C for 24 hrs after the autoclave cycles. These BIs included unautoclaved positive control indicators.

Autoclave Cycles: Two autoclave cycles were tested. First cycle test: PreVac 4-pulse, 121°C-40-minute sterilization with 5-minute dry. The second cycle test: PreVac 4-pulse, 121°C-30-minute sterilization with 5-minute dry.

Tissues used for testing: The simulated carcass mass was composed of beef bottom roast cut into 4 inch to 6 inch strips, averaging 2 inch x 2 inch thickness. These simulated carcasses

were refrigerated to a temperature of 5°C/41°F and this was the starting temperature at the initiation of the autoclave cycle.

Tissue Distribution in Packages: The average weight per simulated carcass mass was 2.8 lbs of simulated (beef roast) carcasses placed into sealable ziplock plastic bags.

Test Procedures:

For Test #1 and Test #2 the autoclave times were set as 40-minute cycles using a single Safe-Decon container with NO autoclavable bag lining the basket. Each test used two biologicals, one put into the center of a bag of simulated carcasses (2.6 lbs & 2.7 lbs of beef roast) inside a sealable plastic bag and the second indicator was placed in the liquid collection area. An autoclave reference load probe was installed inside the bag of tissue. The Safe-Decon container was placed on the lowest level of the cart over the drain of the autoclave. After the cycle, the indicators were removed and incubated as per the manufacturer's instructions (60°C for 24 hours). Total cycle time for Test #1 = 72 minutes and for Test #2 = 67 minutes.

For Test #3 (30-minute cycle) two Safe-Decon containers were used with no autoclavable bag lining the baskets, each containing two BIs per container, one BI was placed into the center of a bag of simulated carcasses (2.6 lbs) within a sealable Ziplock plastic bag and the second indicator was placed in the liquid collection area. A reference load probe was installed

inside the bag of meat of container #1, the second container (marked as #4) had two indicators, both within the bag of 2.12 lbs of beef (located in the top and bottom of the bag). The containers were placed on the lowest level of the cart over the drain of the autoclave. After the cycle the indicators were removed and incubated as per the manufacturer's instructions (60°C for 24 hours).

Test Results:

Test Results	Insert date of read here->	10 APR 23 0303 hrs and run time 72 mins			
Test #1		Results (POS or NEG)			
	Placement	Beef 1		Beef 2	
Validation Test BIs	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon				
(+) Control	Not autoclaved	POS			
Autoclave Run Time/Temps		Time: 40.01 mins at Temps: 124.8°C to 124.8°C			

Test Results	Insert date of read here->	10 APR 23 1629 hr and run time = 67 mins			
Test # 2		Results (POS or NEG)			
	Placement	Beef 1		Beef 2	
Validation Test BIs	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			

	Within bag in liquid catch area	NEG
	Outside Safe-Decon	NEG
(+) Control	Not autoclaved	POS
Autoclave Run Time/Temps		Time: 40.1 mins at 121°C to 124.8°C

Test Results	Insert date of read here->	10 APR 23 1757 hrs and run time = 56 - 63 mins			
Test # 3 SD#1		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test BIs	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NEG			
(+) Control	Not autoclaved	POS			
Autoclave Run Time/Temps		Time: 30.23 mins at 122.0°C to 124.9°C			

Test Results	Insert date of read here->	10 APR 23 1757 hrs and run time = 56 - 63 mins			
Test # 3 SD#2		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test BIs	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			

	Outside Safe-Decon	NEG
(+) Control	Not autoclaved	POS
Autoclave Run Time/Temps		Time: 30.23 mins at 122.0°C to 124.9°C

BI Indicator Results - Test #1 and Test #2 after 24 hr incubation

	Tissue	Between Autoclave Bags	Liquid	Control	Ref. Load Probe	Time	
Test #1	NEG	NEG	NEG	POS	ND	72 mins	
Test #2	NEG	NEG	NEG	POS	ND	67 mins	
Test #3 SD#1	NEG	NA	NEG	POS		56 – 63 mins	
Test #3 SD#2	Top – NEG Bottom – NEG	NA	ND	POS		56 – 63 mins	

BI indicator incubation: NEG = negative culture; POS – positive culture; SD – SafeDecon; ND – No Data; NA – Not applicable; Ref. Load Probe – Tuttanauer autoclave load probe

Test #1 and Test #2: BI Results



Test #3: SD#1 Load 1 containing BIs #1 and BI#2 and SD#2 Load 2 containing BIs #3 and BI #4



Photos of BI Placement and Container Location in Autoclave



Placement of Reference Probe



Location of SafeDecon Container in Autoclave



Location of BI After Autoclave Cycle



Procedure for the 2nd series of Testing:

Tissue: 15 lbs portioned into 2-2lbs 8oz (5lbs total) double bagged beef round roast. Tissue is divided into 5" to 6" 2X2 strips.

BI Indicators: CROSSTEC Spore Ampule Biological Indicator (BI) 10/6 Lot# S2120506, Exp Date: 2024-03-31. After completion of an autoclave cycle the BIs were incubated as per the manufacturer's instructions (60°C for 24- 48 hours).

Autoclave: Tuttnauer 69137 Single door autoclave.

Placement of BIs: A cut was made to the center of a 6oz of meat and a CROSSTEX BI was placed inside the tissue and the opening was sealed with a tissue plug. A second CROSSTEX BI was placed into the liquid catchment area. A MesaLabs EZ test

BI was placed between the bag and Safe-Decon inner container. The SafeDecon container was then placed in the lower corner of the autoclave.

Autoclave Settings: 1 minute purge, 4 PreVac pulses, 30-minute sterilization and a 5-minute dry. The total cycle time for this study was 63 minutes.

Crosstec BI within Tissue and Closed with Tissue Plug



Crosstec BI on the exterior of the autoclave bag



Test Tissue in Sealable Bags



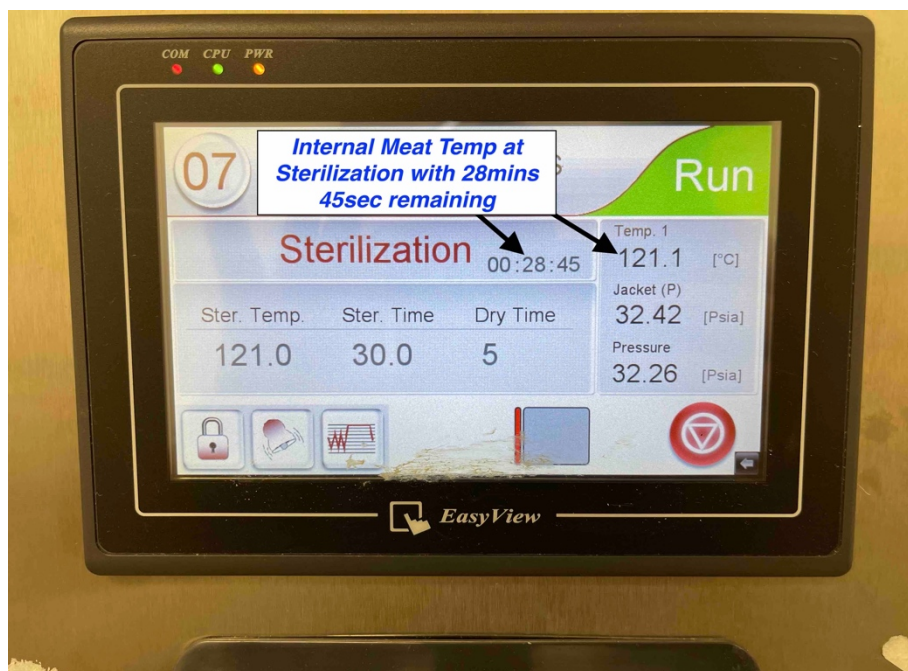
Location of SafeDecon Container in Autoclave



Tissue Temperature using Reference Probe before Autoclaving



Tissue Temperature During Autoclaving



Tissue after autoclaving



BIs Incubated at 60°C for 48 hrs



Autoclave Data for Tests

Test #1

Test #2

Test #3

Date: 10/APR/2023
Time: 03:03:53
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 16
Cycle Num: 001769
30min Prevac 10Dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 40.0 min*
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 045.7 14.42
P 00:01:07 106.3 22.19
A 00:01:08 106.9 22.48
A 00:03:04 093.7 09.66
A 00:04:15 115.5 26.85
A 00:07:15 098.9 11.89
A 00:08:17 084.9 06.96
A 00:09:55 119.2 28.47
A 00:12:55 089.7 08.19
A 00:13:13 085.0 06.98
A 00:14:46 119.3 28.61
A 00:17:47 085.4 07.08
H 00:20:06 119.9 28.61
H 00:21:22 121.5 30.86
CLK 1: 03:25:16
CLK 2: 03:25:16
S 00:21:23 121.8 31.15
S 00:22:23 124.7 32.75
S 00:23:23 124.7 32.65
S 00:24:23 124.8 32.72
S 00:25:23 124.8 32.73
S 00:26:23 125.0 32.88
S 00:27:23 124.8 32.69
S 00:28:23 124.8 32.66
S 00:29:23 124.6 32.62
S 00:30:23 124.8 32.81
S 00:31:23 124.9 32.73
S 00:32:23 124.8 32.62
S 00:33:23 124.8 32.66
S 00:34:23 125.0 32.92
S 00:35:23 124.8 32.65
S 00:36:23 124.7 32.81
S 00:37:23 124.8 32.60
S 00:38:23 124.8 32.65
S 00:39:23 124.8 32.72
S 00:40:23 124.8 32.66
S 00:41:23 124.8 32.65
S 00:42:23 124.8 32.78
S 00:43:23 124.8 32.60
S 00:44:23 124.8 32.65
S 00:45:23 124.8 32.65
S 00:46:23 124.8 32.60
S 00:47:23 124.8 32.57
S 00:48:23 124.7 32.60
S 00:49:23 124.8 32.69
S 00:50:23 124.8 32.92
S 00:51:23 124.7 32.78
S 00:52:23 124.8 32.59
S 00:53:23 124.7 32.62
S 00:54:23 124.7 32.76
S 00:55:23 124.7 32.59
S 00:56:23 124.7 32.59
S 00:57:23 124.7 32.68
S 00:58:23 124.8 32.59
S 00:59:23 124.8 32.76
S 01:00:23 124.8 32.69
S 01:01:23 124.8 32.65
S 01:01:24 124.8 32.62
CLK 1: 04:05:17
CLK 2: 04:05:17
E 01:01:25 124.8 32.62
E 01:02:29 105.1 15.84
D 01:02:30 105.1 15.84
D 01:05:30 068.9 01.89
D 01:07:31 075.9 01.60
01:07:35 076.0 01.60
01:09:00 074.6 13.73
Status: Cycle Ended
Time: 04:12:53
Operator: _____
=====

Date: 10/APR/2023

Test #4

Date: 10/APR/2023
Time: 04:29:09
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 16
Cycle Num: 001770
30min Prevac 10Dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 40.0 min*
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 055.7 14.40
P 00:01:32 112.7 23.25
A 00:01:32 112.9 23.55
A 00:04:01 092.6 09.50
A 00:05:00 114.3 27.02
A 00:08:00 091.5 09.30
A 00:08:32 084.0 06.98
A 00:10:32 119.7 28.35
A 00:13:32 086.1 07.50
A 00:13:38 084.9 07.08
A 00:15:18 118.6 28.50
A 00:18:12 085.3 07.11
H 00:19:32 117.5 28.37
H 00:20:48 121.9 31.33
CLK 1: 04:49:58
CLK 2: 04:49:58
S 00:20:50 121.9 31.33
S 00:21:50 124.6 32.54
S 00:22:50 124.7 32.69
S 00:23:50 124.6 32.59
S 00:24:50 124.7 32.73
S 00:25:50 124.8 32.59
S 00:26:50 124.8 32.59
S 00:27:50 124.8 32.54
S 00:28:50 124.9 32.66
S 00:29:50 124.8 32.66
S 00:30:50 124.8 32.62
S 00:31:50 124.8 32.59
S 00:32:50 124.7 32.69
S 00:33:50 124.8 32.59
S 00:34:50 124.8 32.69
S 00:35:50 124.7 32.54
S 00:36:50 124.7 32.62
S 00:37:50 124.8 32.59
S 00:38:50 124.7 32.75
S 00:39:50 124.8 32.59
S 00:40:50 124.8 32.60
S 00:41:50 124.8 32.59
S 00:42:50 124.6 32.59
S 00:43:50 124.7 32.98
S 00:44:50 124.8 32.60
S 00:45:50 124.7 32.57
S 00:46:50 124.9 32.78
S 00:47:50 124.7 32.69
S 00:48:50 124.8 32.68
S 00:49:50 124.7 32.75
S 00:50:50 124.7 32.66
S 00:51:50 124.7 32.66
S 00:52:50 124.7 32.53
S 00:53:50 124.7 32.73
S 00:54:50 124.8 32.69
S 00:55:50 124.9 32.75
S 00:56:50 124.8 32.81
S 00:57:50 124.8 32.59
S 00:58:50 124.7 32.98
S 00:59:50 124.8 32.59
S 01:00:50 124.8 32.60
S 01:00:50 124.8 32.60
CLK 1: 05:29:59
CLK 2: 05:29:58
E 01:00:51 124.8 32.60
E 01:01:52 104.9 15.76
D 01:01:52 104.9 15.76
D 01:04:52 074.3 02.03
D 01:06:53 078.5 01.57
01:06:57 078.7 01.55
01:08:22 076.3 13.73
Status: Cycle Ended
Time: 05:37:32
Operator: _____
=====

Test #5

Date: 10/APR/2023.
Time: 05:57:21
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 15
Cycle Num: 001771
30min Prevac 10Dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 056.0 14.36
P 00:01:30 113.0 23.13
A 00:01:30 113.0 23.13
A 00:03:56 092.3 09.38
A 00:05:00 115.7 27.05
A 00:08:00 092.2 09.47
A 00:08:34 083.4 06.82
A 00:10:23 119.5 28.45
A 00:13:23 087.8 07.90
A 00:13:38 084.4 06.89
A 00:15:25 119.1 28.35
A 00:18:25 084.1 06.79
H 00:20:16 119.5 28.60
H 00:21:31 121.7 31.24
CLK 1: 06:18:53
CLK 2: 06:18:53
S 00:21:32 122.0 31.54
S 00:22:32 124.7 32.65
S 00:23:32 124.7 32.72
S 00:24:32 124.7 32.99
S 00:25:32 124.6 32.76
S 00:26:32 124.8 32.68
S 00:27:32 124.8 32.75
S 00:28:32 124.9 32.66
S 00:29:32 124.9 32.62
S 00:30:32 124.8 32.60
S 00:31:32 124.8 32.68
S 00:32:32 124.8 32.62
S 00:33:32 124.8 32.57
S 00:34:32 124.9 32.62
S 00:35:32 124.9 32.82
S 00:36:32 124.9 32.83
S 00:37:32 124.8 32.60
S 00:38:32 124.8 32.99
S 00:39:32 124.7 32.60
S 00:40:32 124.8 32.60
S 00:41:32 124.8 32.76
S 00:42:32 124.9 32.62
S 00:43:32 124.9 32.75
S 00:44:32 124.8 32.75
S 00:45:32 124.9 32.66
S 00:46:32 124.6 32.83
S 00:47:32 124.8 32.82
S 00:48:32 124.7 32.72
S 00:49:32 124.8 32.65
S 00:50:32 124.7 33.04
S 00:51:32 124.9 32.78
S 00:51:33 124.9 32.76
CLK 1: 06:48:54
CLK 2: 06:48:54
E 00:51:34 124.9 32.76
E 00:52:37 104.9 15.74
D 00:52:38 104.3 15.74
D 00:55:38 067.8 02.26
D 00:57:38 077.2 01.64
00:57:42 077.2 01.62
00:59:08 073.8 13.71
Status: Cycle Ended
Time: 06:56:29
Operator: _____
=====

Test #6

Date: 31/MAR/2023
Time: 05:20:05
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 13
Cycle Num: 001759
30min Prevac 100dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 10.0 min
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 078.7 14.55
P 00:01:07 114.1 22.71
A 00:01:07 114.5 22.97
A 00:04:03 095.6 09.70
A 00:04:45 110.4 22.07
A 00:07:29 090.9 07.93
A 00:08:11 108.9 21.78
A 00:11:12 082.9 05.73
H 00:12:06 111.8 23.80
H 00:15:06 119.5 24.92
H 00:18:06 120.4 25.74
H 00:21:06 118.3 25.28
H 00:24:06 120.0 28.86
H 00:24:25 121.5 30.30
CLK 1: 05:44:31
CLK 2: 05:44:31
S 00:24:26 121.8 30.53
S 00:25:26 124.8 32.75
S 00:26:26 124.9 32.85
S 00:27:26 124.9 32.68
S 00:28:26 124.9 32.60
S 00:29:26 124.9 32.91
S 00:30:26 125.0 32.59
S 00:31:26 124.9 32.66
S 00:32:26 124.9 32.60
S 00:33:26 125.0 32.72
S 00:34:26 125.1 32.65
S 00:35:26 124.8 32.78
S 00:36:26 124.9 32.82
S 00:37:26 124.9 32.69
S 00:38:26 125.1 32.72
S 00:39:26 124.9 32.59
S 00:40:26 124.9 32.68
S 00:41:26 125.0 32.60
S 00:42:26 124.9 32.81
S 00:43:26 124.9 32.69
S 00:44:26 125.0 32.68
S 00:45:26 125.0 32.85
S 00:46:26 125.1 32.82
S 00:47:26 125.0 32.68
S 00:48:26 125.0 32.66
S 00:49:26 125.0 32.73
S 00:50:26 124.9 32.60
S 00:51:26 124.9 32.92
S 00:52:26 124.9 32.60
S 00:53:26 125.0 32.92
S 00:54:26 125.1 32.75
S 00:54:26 125.1 32.75
CLK 1: 06:14:31
CLK 2: 06:14:31
E 00:54:27 125.1 32.73
E 00:55:42 104.3 15.72
D 00:55:42 104.3 15.72
D 00:58:42 088.8 01.65
D 01:01:42 084.6 01.39
D 01:04:42 079.8 01.39
D 01:05:44 078.4 01.38
01:05:48 078.4 01.35
01:07:10 074.5 13.78
Status: Cycle Ended
Time: 06:27:15
Operator: _____

Date: 31/MAR/2023
Time: 06:46:00
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 13
Cycle Num: 001760
30min Prevac 100dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 10.0 min
End Temp: 120.0 °C
Time °C Psia
P 00:00:05 053.4 14.75
P 00:01:35 114.0 23.83
A 00:01:35 114.0 23.83
A 00:04:10 092.3 09.47
A 00:04:51 107.3 22.12
A 00:07:34 088.0 07.93
A 00:08:19 105.7 22.44
A 00:11:19 082.6 06.34
A 00:11:29 080.5 05.82
H 00:12:26 109.2 24.26
H 00:14:20 121.4 30.41
CLK 1: 07:00:21
CLK 2: 07:00:21
S 00:14:21 121.7 30.66
S 00:15:21 124.8 32.91
S 00:16:21 124.8 32.59
S 00:17:21 124.8 32.72
S 00:18:21 125.1 32.76
S 00:19:21 124.8 32.78
S 00:20:21 125.1 32.89
S 00:21:21 125.0 32.76
S 00:22:21 125.0 32.62
S 00:23:21 125.1 32.69
S 00:24:21 125.0 32.73
S 00:25:21 124.9 32.60
S 00:26:21 124.8 32.75
S 00:27:21 125.0 32.65
S 00:28:21 124.9 32.62
S 00:29:21 124.9 32.66
S 00:30:21 124.9 32.60
S 00:31:21 124.9 32.66
S 00:32:21 124.9 32.62
S 00:33:21 125.0 32.72
S 00:34:21 124.9 32.66
S 00:35:21 124.9 32.62
S 00:36:21 124.9 32.66
S 00:37:21 124.8 32.73
S 00:38:21 124.8 32.68
S 00:39:21 124.9 32.60
S 00:40:21 124.9 32.65
S 00:41:21 124.9 32.62
S 00:42:21 124.8 32.81
S 00:43:21 124.8 32.60
S 00:44:21 125.0 32.60
S 00:44:21 125.0 32.60
CLK 1: 07:30:21
CLK 2: 07:30:21
E 00:44:22 125.0 32.60
E 00:45:37 104.5 15.75
D 00:45:37 104.5 15.75
D 00:48:37 089.3 01.86
D 00:51:37 086.2 01.44
D 00:54:37 082.2 01.39
D 00:55:38 081.0 01.39
00:55:42 080.9 01.39
00:57:06 076.8 13.81
Status: Cycle Ended
Time: 07:43:06
Operator: _____

Date: 31/MAR/2023
Time: 08:00:55
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 13
Cycle Num: 001761
30min Prevac 100dry
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 10.0 min
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 055.8 14.89
P 00:01:30 112.1 23.39
A 00:01:30 112.3 23.39
A 00:04:01 093.0 09.63
A 00:04:37 104.0 21.77
A 00:07:07 088.1 07.73
A 00:07:49 104.5 21.96
A 00:10:37 080.8 05.89
H 00:11:31 107.4 23.42
H 00:13:34 121.4 30.64
CLK 1: 08:14:30
CLK 2: 08:14:30
S 00:13:36 121.4 30.64
S 00:14:36 124.9 33.01
S 00:15:36 124.9 32.76
S 00:16:36 124.8 32.66
S 00:17:36 124.8 32.69
S 00:18:36 124.9 32.85
S 00:19:36 125.0 32.66
S 00:20:36 125.1 32.82
S 00:21:36 125.0 32.66
S 00:22:36 125.0 32.62
S 00:23:36 125.0 32.69
S 00:24:36 125.0 32.78
S 00:25:36 125.0 32.85
S 00:26:36 124.8 32.76
S 00:27:36 124.9 32.73
S 00:28:36 125.0 32.66
S 00:29:36 124.8 32.54
S 00:30:36 124.8 32.69
S 00:31:36 124.9 32.54
S 00:32:36 124.8 32.54
S 00:33:36 124.8 32.60
S 00:34:36 125.0 32.69
S 00:35:36 124.8 32.65
S 00:36:36 124.9 32.60
S 00:37:36 124.8 32.60
S 00:38:36 124.9 32.57
S 00:39:36 124.8 32.53
S 00:40:36 124.9 32.66
S 00:41:36 124.9 32.72
S 00:42:36 124.9 32.60
S 00:43:36 124.9 32.54
S 00:43:36 124.9 32.54
CLK 1: 08:44:31
CLK 2: 08:44:31
E 00:43:37 124.9 32.54
E 00:44:45 104.6 15.75
D 00:44:46 104.6 15.75
D 00:47:46 087.8 01.77
D 00:50:46 086.5 01.51
D 00:53:46 082.5 01.51
D 00:54:47 081.0 01.51
00:54:51 080.9 01.51
00:56:14 077.2 13.72
Status: Cycle Ended
Time: 08:57:09
Operator: _____

Test #7

Test #8

Date: 13/APR/2023
Time: 04:30:40
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 16
Cycle Num: 001773
30min Prevac 10Dry
(Customized)
Ster. Temp. 121.0 ~°C
Ster. Time 40.0 min*
Dry Time 5.0 min*
End Temp: 120.0 ~°C
Time ~°C Psia
P 00:00:06 043.6 14.31
P 00:01:07 104.6 22.26
A 00:01:07 104.6 22.26
A 00:03:02 093.3 09.46
A 00:04:17 115.7 26.82
A 00:07:17 103.6 15.62
A 00:08:45 084.7 06.96
A 00:10:56 119.6 28.43
A 00:13:56 097.9 11.54
A 00:14:50 085.0 06.98
A 00:16:26 119.2 28.45
A 00:19:26 091.4 08.92
A 00:19:52 084.7 06.96
H 00:22:16 119.8 28.37
H 00:23:33 121.6 31.01
CLK 1: 04:54:14
CLK 2: 04:54:14
S 00:23:34 121.6 31.01
S 00:24:34 124.6 32.73
S 00:25:34 124.7 32.72
S 00:26:34 124.8 33.08
S 00:27:34 124.7 32.59
S 00:28:34 124.8 32.65
S 00:29:34 124.7 32.68
S 00:30:34 124.8 32.76
S 00:31:34 124.8 32.65
S 00:32:34 124.8 32.73
S 00:33:34 124.8 32.66
S 00:34:34 124.8 32.82
S 00:35:34 124.7 32.75
S 00:36:34 124.8 32.68
S 00:37:34 124.8 32.72
S 00:38:34 124.8 32.69
S 00:39:34 124.7 32.33
S 00:40:34 124.8 32.66
S 00:41:34 124.9 32.62
S 00:42:34 124.8 32.65
S 00:43:34 124.8 32.72
S 00:44:34 124.9 32.82
S 00:45:34 124.8 32.60
S 00:46:34 124.8 32.62
S 00:47:34 124.8 32.83
S 00:48:34 124.8 32.73
S 00:49:34 124.8 32.68
S 00:50:34 124.9 32.73
S 00:51:34 124.8 32.72
S 00:52:34 124.7 32.75
S 00:53:34 124.8 32.72
S 00:54:34 124.8 32.69
S 00:55:34 124.9 32.75
S 00:56:34 124.8 32.66
S 00:57:34 124.7 32.69
S 00:58:34 124.7 32.73
S 00:59:34 124.8 32.65
S 01:00:34 124.8 32.76
S 01:01:34 124.8 32.75
S 01:02:34 124.8 32.76
S 01:03:34 124.7 32.82
S 01:03:34 124.7 32.82
CLK 1: 05:34:14
CLK 2: 05:34:13
E 01:03:35 124.8 32.81
E 01:04:43 104.7 15.79
D 01:04:44 104.7 15.79
D 01:07:44 088.9 01.94
D 01:09:45 087.9 01.57
01:09:49 087.8 01.58
01:11:15 083.8 13.71
Status: Cycle Ended
Time: 05:41:55
Operator: _____

Date: 13/APR/2023
Time: 06:00:45
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 15
Cycle Num: 001774
30min Prevac 10Dry
(Customized)
Ster. Temp. 121.0 ~°C
Ster. Time 30.0 min*
Dry Time 5.0 min*
End Temp: 120.0 ~°C
Time ~°C Psia

P 00:00:07 056.6 14.24
P 00:01:31 112.9 22.90
A 00:01:32 113.2 23.16
A 00:04:03 092.0 09.25
A 00:05:07 115.7 26.87
A 00:08:07 088.5 07.82
A 00:08:19 085.3 07.05
A 00:09:53 119.3 28.61
A 00:12:53 087.6 07.69
A 00:13:04 085.4 07.08
A 00:14:23 118.4 28.37
A 00:17:19 085.8 07.12
H 00:19:17 119.7 28.50
H 00:20:21 121.4 30.66
CLK 1: 06:21:07
CLK 2: 06:21:06
S 00:20:22 121.7 30.99
S 00:21:22 124.7 32.59
S 00:22:22 124.7 32.78
S 00:23:22 124.8 32.76
S 00:24:22 124.7 32.69
S 00:25:22 124.8 32.83
S 00:26:22 124.9 32.85
S 00:27:22 124.8 32.76
S 00:28:22 124.9 32.60
S 00:29:22 124.9 32.69
S 00:30:22 124.9 32.73
S 00:31:22 124.9 32.68
S 00:32:22 124.9 32.66
S 00:33:22 124.9 32.66
S 00:34:22 124.9 32.73
S 00:35:22 124.9 32.60
S 00:36:22 124.8 32.65
S 00:37:22 125.0 32.78
S 00:38:22 124.9 32.60
S 00:39:22 124.9 32.68
S 00:40:22 124.8 32.88
S 00:41:22 124.9 32.62
S 00:42:22 124.8 32.62
S 00:43:22 124.9 32.66
S 00:44:22 124.8 32.75
S 00:45:22 124.8 32.65
S 00:46:22 124.9 32.62
S 00:47:22 125.1 32.81
S 00:48:22 124.9 32.69
S 00:49:22 124.8 32.85
S 00:50:22 124.8 32.72
S 00:50:22 124.8 32.72
CLK 1: 06:51:07
CLK 2: 06:51:06
E 00:50:23 124.8 32.76
E 00:51:29 104.4 15.72
D 00:51:29 104.4 15.72
D 00:54:29 090.5 01.90
D 00:56:30 089.4 01.48
00:56:35 089.3 01.46
00:58:02 085.8 13.73
Status: Cycle Ended
Time: 06:58:47
Operator: _____

Test #9

Test #10

Test #11

Date: 16/MAY/2023
Time: 04:10:20
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 15
Cycle Num: 001798
SAFEDECON CARCS
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 051.1 15.47
P 00:01:31 112.0 24.87
A 00:01:32 112.5 24.89
A 00:04:32 087.3 08.61
A 00:04:43 084.9 07.99
A 00:05:51 114.6 26.85
A 00:08:51 086.3 08.25
A 00:10:45 065.6 04.09
A 00:12:48 119.0 28.51
A 00:15:48 081.0 06.80
A 00:17:02 064.8 04.05
A 00:18:33 119.0 28.56
A 00:21:33 079.0 06.44
A 00:22:38 065.0 04.08
H 00:24:11 118.6 28.57
H 00:25:33 121.7 31.88
CLK 1: 04:35:54
CLK 2: 04:35:54
S 00:25:34 122.1 32.21
S 00:26:34 124.3 32.50
S 00:27:34 123.6 31.88
S 00:28:34 123.5 31.91
S 00:29:34 123.5 31.98
S 00:30:34 123.9 32.27
S 00:31:34 123.5 31.96
S 00:32:34 123.8 32.25
S 00:33:34 123.6 31.96
S 00:34:34 123.8 31.95
S 00:35:34 123.9 32.20
S 00:36:34 123.6 32.04
S 00:37:34 123.7 31.89
S 00:38:34 123.7 31.86
S 00:39:34 123.8 31.94
S 00:40:34 123.7 32.04
S 00:41:34 123.8 31.98
S 00:42:34 123.8 32.02
S 00:43:34 123.7 31.86
S 00:44:34 123.8 31.89
S 00:45:34 123.7 31.91
S 00:46:34 123.8 31.89
S 00:47:34 123.8 31.99
S 00:48:34 123.6 31.98
S 00:49:34 123.7 31.88
S 00:50:34 123.8 31.89
S 00:51:34 123.7 31.89
S 00:52:34 123.9 32.21
S 00:53:34 123.7 31.88
S 00:54:34 123.6 32.12
S 00:55:34 123.7 31.83
S 00:55:34 123.7 31.83
CLK 1: 05:05:54
CLK 2: 05:05:54
E 00:55:35 123.6 31.86
E 00:56:38 105.6 16.85
D 00:56:39 105.6 16.85
D 00:59:39 094.5 03.02
D 01:01:40 092.7 02.54
D 01:01:44 092.6 02.54
01:03:09 087.7 14.84
Status: Cycle Ended
Time: 05:13:30
Operator: _____

Date: 15/MAY/2023
Time: 04:12:59
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 15
Cycle Num: 001795
SAFEDECON CARCS
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:05 044.3 15.59
P 00:01:07 110.9 22.80
A 00:01:07 110.9 22.80
A 00:03:47 084.8 07.86
A 00:05:17 116.9 26.89
A 00:08:17 104.6 16.94
A 00:11:05 073.2 05.11
A 00:12:45 118.2 28.47
A 00:15:45 094.7 10.86
A 00:17:16 072.8 05.02
A 00:18:51 118.2 28.37
A 00:21:51 088.2 08.64
A 00:22:55 073.2 05.16
H 00:24:33 118.4 28.40
H 00:25:46 121.6 31.36
CLK 1: 04:38:46
CLK 2: 04:38:46
S 00:25:47 121.6 31.36
S 00:26:47 123.9 32.10
S 00:27:47 123.7 31.95
S 00:28:47 123.8 31.86
S 00:29:47 123.7 31.96
S 00:30:47 123.7 31.99
S 00:31:47 123.8 31.98
S 00:32:47 123.8 32.18
S 00:33:47 123.9 32.21
S 00:34:47 123.9 32.04
S 00:35:47 123.7 32.11
S 00:36:47 123.7 31.83
S 00:37:47 123.7 31.94
S 00:38:47 124.0 32.28
S 00:39:47 123.8 31.86
S 00:40:47 123.9 32.05
S 00:41:47 123.8 31.94
S 00:42:47 123.8 31.89
S 00:43:47 123.7 32.11
S 00:44:47 123.9 31.96
S 00:45:47 123.9 31.91
S 00:46:47 123.9 32.02
S 00:47:47 123.9 31.96
S 00:48:47 123.7 31.88
S 00:49:47 123.8 31.99
S 00:50:47 123.8 31.94
S 00:51:47 123.8 32.10
S 00:52:47 123.9 32.02
S 00:53:47 123.9 32.02
S 00:54:47 123.9 32.02
S 00:55:47 123.8 32.11
S 00:55:47 123.8 32.11
CLK 1: 05:08:46
CLK 2: 05:08:45
E 00:55:48 123.8 32.10
E 00:56:50 105.9 16.93
D 00:56:50 105.9 16.93
D 00:59:50 090.3 02.96
D 01:01:51 088.8 02.60
D 01:01:55 088.7 02.60
01:03:20 084.9 14.82
Status: Cycle Ended
Time: 05:16:19
Operator: _____

Date: 16/MAY/2023
Time: 02:51:33
Ser. Num: 1208053
Model: 69137 1R SP BH
Version: 15
Cycle Num: 001797
SAFEDECON CARCS
(Customized)
Ster. Temp. 121.0 °C
Ster. Time 30.0 min
Dry Time 5.0 min*
End Temp: 120.0 °C
Time °C Psia
P 00:00:06 045.8 15.45
P 00:01:07 111.0 24.25
A 00:01:07 111.0 24.25
A 00:03:51 085.2 07.85
A 00:05:11 115.9 26.77
A 00:08:11 092.1 09.86
A 00:10:24 066.0 04.08
A 00:12:11 118.5 28.57
A 00:15:11 088.8 08.86
A 00:17:06 065.8 04.05
A 00:18:47 118.6 28.54
A 00:21:47 082.5 07.06
A 00:23:11 066.1 04.12
H 00:24:49 118.2 28.44
H 00:26:07 121.8 31.72
CLK 1: 03:17:41
CLK 2: 03:17:40
S 00:26:08 121.8 31.72
S 00:27:08 124.3 32.44
S 00:28:08 123.7 31.98
S 00:29:08 123.8 32.28
S 00:30:08 123.7 31.99
S 00:31:08 123.6 31.83
S 00:32:08 123.6 31.86
S 00:33:08 123.7 32.07
S 00:34:08 123.8 31.86
S 00:35:08 123.6 32.02
S 00:36:08 123.8 31.91
S 00:37:08 123.7 31.99
S 00:38:08 123.7 31.86
S 00:39:08 123.8 32.07
S 00:40:08 123.8 31.95
S 00:41:08 123.8 31.88
S 00:42:08 123.7 31.89
S 00:43:08 123.7 31.89
S 00:44:08 123.8 31.86
S 00:45:08 123.8 31.96
S 00:46:08 123.9 32.04
S 00:47:08 123.8 31.96
S 00:48:08 123.6 31.95
S 00:49:08 123.8 31.89
S 00:50:08 123.9 31.96
S 00:51:08 123.8 31.96
S 00:52:08 123.7 31.89
S 00:53:08 123.7 31.83
S 00:54:08 123.8 31.99
S 00:55:08 123.8 31.94
S 00:56:08 123.7 31.88
S 00:56:08 123.7 31.88
CLK 1: 03:47:41
CLK 2: 03:47:41
E 00:56:09 123.7 31.86
E 00:57:15 105.7 16.84
D 00:57:15 105.7 16.84
D 01:00:15 059.2 03.15
D 01:02:16 057.1 02.73
D 01:02:21 057.3 02.71
01:03:45 058.3 14.85
Status: Cycle Ended
Time: 03:55:18
Operator: _____

Results

Test Results	Insert date of read here->	31 MAR 23 0520 hrs			
Test #4		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	31 MAR 23 0646 hrs			
Test #5		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	31 MAR 23 0800 hrs			
Test #6		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	13 APR 23 0410 hrs			
Test #7		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	13 APR 23 0600 hrs			
Test #8		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			

	Within bag in liquid catch area	NEG
	Outside Safe-Decon	NA
(+) Control	Not autoclaved	POS

Test Results	Insert date of read here->	16 MAY 23 0410 hrs			
Test #9		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	15 MAY 23 0412 hrs			
Test #10		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

Test Results	Insert date of read here->	16 MAY 23 0251 hrs			
Test #11		Results (+or-)			
	Placement	Beef 1		Beef 2	
Validation Test Bls	Within "carcass"	NEG		NEG	
Standard locations	Between bag and Safe-Decon 200	NEG			
	Within bag in liquid catch area	NEG			
	Outside Safe-Decon	NA			
(+) Control	Not autoclaved	POS			

CONCLUSION

After configuring the autoclave to allow real time monitoring of the temperatures inside the autoclave, tissue and container, it was concluded that the liquids mixing in with the tissue created an insulating effect. It was noted that the temperatures would fluctuate and never were consistent during the sterilization, with temperature decreases of up to 15°C-20°C which would last for up to 15 minutes. Consequently, the tissue and the liquids needed to be allowed to separate during the sterilization phase of the cycle. After repeating this experiment 11 times forcing the liquids to separate from the tissue using 4 Prevac pulses, the temperature results were consistent, stable, and reproducible. It was repeatedly demonstrated that the tissue temperature increase remained consistent and reproducible. It was noted that as the liquid was forced out of the tissue by Prevac pulses and separated from the tissue the temperature would rise more rapidly at the tissue core. We concluded this is the reason that a 4-Pre-Vac phase is important with the Safe-Decon process, the first pulse would cause the seals to open and permitting steam, pressure and heat to directly contact the tissue. The follow-on 3 pulses

facilitate pressure and heat penetration deep into the tissue thus forcing out any remaining liquid. The extrusion of the liquid permits the internal temperatures to achieve sterilization temperatures.

At the conclusion of the cycle, it was noted that the tissue was dry and crumbled upon manipulation. The liquid that was collected in the bottom of the container achieved the necessary 121°C/250°F sterilization at the same time as the tissue, as noted by consistent negative Biological Indicators for all tissues and liquids.

Therefore, the SafeDecon container system reliably, efficiently and completely sterilizes bulk tissue and can be used to decontaminate infected experimental murine carcasses from BSL-3/4 laboratories in two bags weighing 2.8 oz each (total 5 lbs) in 30 mins using the autoclave setting parameters outline in this document.