Evacle
Bio Medical Waste & Healthcare Solutions

Evacle 80
Medical Waste Treatment Unit
Validation Report

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Disclosure

The Efficacy Test Validation was performed with the professional guidance of ACCREDITED LABORATORY, OECD-GLP & FDA accredited laboratory:

The Liquid Drainage Composition Validation tests were performed by Accredited Laboratory.

This report is based on ACCREDITED LABORATORY protocol and result certificates with an intention to give the reader a clarified view and comprehensive understanding of the validation process.

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Abstract

Health care waste is increasingly generated worldwide. The Evacle unit was designed to treat infectious healthcare waste on-site at the place of generation by simultaneously shredding and sterilizing the waste through chemical treatment. The sterilant used is branded as Evacetic and it is based on the synergistic sterilizing effects of peracetic acid (PAA) and hydrogen peroxide (HP).

This research was conducted in order to test and approve the efficacy of the biocide alongside with the activity of the Evacle 80 unit. To this end, a surrogated healthcare waste was inoculated with 10^6 CFU/ml of the biological indicator bacteria *Geobacilus stearothermophilus* spores. The waste was then inserted into the Evacle 80 unit and processed according to the established parameters of the sterilization process. Following the sterilization process, liquid and solid waste samples were analyzed to detect viable spores' presence.

No viable bacteria were found in the samples following sterilization process by the Evacle 80 unit. Sterilization process showed 6 $\log_{10}$ CFU/ml bacterial spores' reduction, which complies with STAATT Level IV, according to the ISTAATT guidelines.

The Challenge in Medical Waste Treatment Validation

Most countries still use incinerators and do not adopt heat treatment technologies, such as autoclaves and microwaves, for on-site medical waste treatment. The medical waste carries both solid and liquid phase and the mix and volume of each varies from batch to batch. This makes the sterilization process almost impossible to validate.

Liquids present some unique challenges. Liquids take longer to sterilize than other media since liquids have a high heat capacity. As opposed to solids, liquids take much longer to heat up and cool down, and as a result the total autoclaving cycle time is increased dramatically.

For successful sterilization, autoclaves need to remove all air from the chamber due to possible hollow spaces inside a load. In the case of solids, steam is the sterilization medium and must penetrate inside the load and contact with all surfaces, which is why all air must be removed. If air were to remain inside the load, that would compromise the steam's ability to reach all surfaces of the load.

Liquids have no hollow spaces and use the steam as a medium to heat up. The actual sterilization takes place by the liquid sterilizing itself due to its increased temperature. Therefore, there is no requirement to remove air from the chamber, but a requirement for a longer duration to ensure the liquid has reached at least 135°C for 20-30 min. Very long duration (>20 min) at a pick temperature of 135-141°C and under pressure can only be supported by heavy-duty off-site autoclaves

As the amount of liquids is unpredicted and may vary from batch to batch, the autoclave user is forced to use longer time sterilization programs which could take hours. Additionally, autoclaves have no temperature indication for the liquid phase and the user has no real indication of the actual liquid phase temperature during the sterilization cycle.

The Evacle 80 unit avoids this problem completely by performing the entire disinfection process in a liquid phase, using a liquid disinfection solution diluted in water.

The Evacle 80 unit shreds the solid waste to a confetti shape which is mixed vigorously with the liquid waste and disinfecting solution creating a homogeneous solid-liquid mixture to be sterilized.

Therefore:

- Liquids pose no problem.
- There is no need to remove the air from the chamber.
- No extreme temperatures, pressure and vacuum are used.
- There are no pauses for cooling within cycles – the next cycle may start immediately.
- Fully validated and reproducible sterilization results.

Abbreviations Used

PAA	–	Paretic Acid
HP	–	Hydrogen Peroxide
CFU	–	Colony-forming Units
AA	–	Acetic Acid
BI	–	Biological Indicators
STAATT	–	State and Territorial Association on Alternative Treatment Technologies
ATS	–	Artificial Test Soil

Efficacy Test Validation - *Geobacilus stearothermophilus* Spores

Method

Introduction

The Evacle 80 unit has been designed specifically for hospitals and healthcare institutions that produce hazardous biomedical waste. The Evacle 80 unit is a sterilizing shredder intended to treat pathogenic biological waste and convert it into a sanitary waste while simultaneously reducing the raw waste volume by up to 80% and turn it un-recognizable. On completion of the sterilization process, liquids are separated from solid waste, and the used, deactivated biocide is disposed into the drainage system. Solids are collected into a trash bin and discarded to the general waste.

Objectives

The objective of this efficacy test validation was to examine the claims presented by the manufacturer regarding the microbial inactivation efficacy of the Evacle 80 unit, accompanied by the sterilant EVACETIC. The manufacturer claims to comply with STAATT Level IV sterilization, which is defined as the reduction of bacterial spores of the biological indicator *Geobacilus stearothermophilus* by 6 $\log_{10}$ CFU/ml. The biological indicator was chosen according to STAATT guidelines.

The procedure was repeated 3 times to allow replication of the test in order to verify and validate the consistency of the results.

References

- ISO 11737-1:2006(E) Sterilization of medical devices – Microbiological methods. Part 1: Determination of population of microorganisms on products
- Current USP, <61>, <62>, Microbiological Examination of Nonsterile Pharmaceutical Products
- Accredited Laboratory SOP 50.WI.106: Bioburden Test – Presterilization Estimation of population of microorganisms in/on Medical Device
- Other specific Accredited Laboratory SOPs

Materials and Methods

Reference Strains – Spore Suspension

Terragene Bionova® commercial spore suspension BT20S/9 *Geobacillus stearothermophylus* ATCC 7953 in concentration of 1.2 x 10^9 CFU/ml (to be diluted to 10^6 CFU/ml as part of the process); Lot # BT20S9072021; Expiration Date: July 2021.

Reference Microorganisms' Verification Counting

Fresh culture was prepared by inoculating Tryptic Soy Broth from a commercially purchased spore suspension and incubating it at 55°C for 18-24 hours placed on a mechanical shaker. The microbial suspension was heat shocked at 95-100°C for 15 minutes, followed by immediate cooling using ice water. Serial dilutions from the suspension were prepared and 1 ml from the appropriate dilution was plated into Petri plates in duplicates, to determine the inoculum size by pour plate method using Tryptic Soy Agar. The agar plates were then incubated at 55°C for up to 3 days. Following incubation, number of colony-forming units was counted and recorded. Final stock concentration was approximately 1.2 x 10^9 CFU/ml.

Surrogate Waste Composition

The simulated medical waste load was constructed to contain 10% w/w organic material and 90% of inorganic waste. The total weight of the sample was 8 kg.

The simulated inorganic material consisted of 7.2 kg clean medical waste components like metals and plastic (1 kg of needles and tubes), which represents the process worst case due to their structure or reaction with the Evacetic (90% of the total weight).

The simulated organic material consisted of 0.8 kg (10% of the total weight) of ATS – artificial test soil, this component was formulated for simulated use soiling of medical devices, for the purpose of conducting cleaning validations and cleaning verifications. The reconstituted ATS test soil contains the following markers: protein, hemoglobin, carbohydrate, lipids, and insoluble fibers.

The total weight of the load was selected to simulate a typical load mix and volume per machine cycle.

Sterilizing Solution

Sterilization is performed by the Evacetic solution. The solution active substance is peracetic acid (PAA), accompanied by hydrogen peroxide. Both substances are strong oxidizers and act synergistically to create high sporicidal activity.

Sterilization Process Parameters

The sterilization process is comprised of the following parameters: Waste Shredding, Disinfecting Solution (EVACETIC), Temperature (°C), Contact Time (minutes), Sterilant Concentration (ppm), and Load Density.

The Evacle 80 unit sterilization process is characterized by the following parameters' values:

- Shredding Time: 3 minutes
- Contact Time (during mixing): 12 minutes
- Solution Temperature: 38-42°C
- Sterilant Concentration: 450-500 ppm PAA (1% from stock 4.5-5% solution)
- Total Diluted Solution Volume: 25 liters
- Load Density: 8 kg (waist weight) / 25 liters (solution volume) giving a load density of 0.32 kg/l.

Test Procedure Description

Prior to test initiation, the Evacle 80 unit was thoroughly cleaned with water, following by an empty sterilization cycle.

8 kg of simulated medical waste were prepared, inoculated with 25 ml spore suspension, in order achieve final spore concentration of 10^6 spores/ml. The waste was loaded into the Evacle 80 unit and an automatic sterilization cycle was initiated according to the parameters detailed above.

Waste water (liquid fraction) was directly collected into 3 sterile 1-liter bottles that were pre-prepared by Accredited Laboratory.

Shredded and sterilized solid waste confetti were collected randomly into 3 sterile bags of ~100 gr each.

The liquid samples were collected from the derange pipe outlet.

The solid samples were collected from the collection bag of the manual waste separator.

Laboratory Procedure Description

Following inoculation and operation of the Evacle 80 unit, 25 liters of water and solid waste were obtained following treatment.

The liquid samples, 3 containers, each containing 1 liter of the test sample (eluted solution post disinfecting procedure) randomly sampled (total of three liters) were received and tested according to Accredited Laboratory relevant SOPs.

Portions of 250 ml of each sample were filtrated through 0.45 μm membrane filter in duplicates and the membranes were washed with 100 ml of sterile 0.9% Saline with 0.1% Tween 80. The membranes were then placed onto Tryptic Soy Agar plates. The plates were incubated at 55°C for up to 3 days. Following incubation, the number of colonies forming units was counted and recorded.

The solid waste samples 3 sterile bags each containing up to 100 grams of the sample randomly sampled (total of ~300 gr) were received and tested according to Accredited Laboratory relevant SOPs To each bag containing the sample, 500 ml of sterile saline with 0.1% Tween 80 was added. Each bag was then manually agitated and rubbed for 3 minutes, forcing the fluid around and in the sample; the rinsing liquid from each bag was then filtered through a 0.45 μm filter membrane. The filter membrane was transferred onto solid Tryptic Soy Agar (TSA) plates. The plates were incubated at 55°C for up to 3 days. Following the incubation, total number of colonies (CFI-J per bag) on each plate was counted and the results were recorded as CFU/gram.

Results

The following incubation CFU from each sample were counted. No bacterial growth occurred at each of the replicate samples.

Experiment	Sample Description	Sample No.	Filtered Solution volume (ml) / weight (gr)	CFU / Sample
1	Liquid waste	1	1000	<1
	Liquid waste	2	1000	<1
	Liquid waste	3	1000	<1
	Solid waste	1	10	<1
	Solid waste	2	10	<1
	Solid waste	3	10	<1
2	Liquid waste	1	1000	<1
	Liquid waste	2	1000	<1
	Liquid waste	3	1000	<1
	Solid waste	1	10	<1
	Solid waste	2	10	<1
	Solid waste	3	10	<1
3	Liquid waste	1	1000	<1
	Liquid waste	2	1000	<1
	Liquid waste	3	1000	<1
	Solid waste	1	10	<1
	Solid waste	2	10	<1
	Solid waste	3	10	<1

Table 1 – Sterilization results of *Geobacilus stearothermophylus* spores following sterilization process with the Evacle 80 unit

Conclusions

Based on the information detailed above, as well as the data presented in Table 1, it can be concluded that the Evacle 80 unit sterilization process accompanied by the use of the disinfectant Evacetic allows the reduction of 6 $\log_{10}$ CFU/ml of bacterial spores both for the solid and liquid waste phases. This reduction complies with STAATT Level 4 according to the State and Territorial Association on Alternate Treatment Technologies (STAATT) guidelines.

Efficacy Test Validation – *Mycobacteria* Suspension Solution

Method

Introduction

The Evacle 80 unit has been designed specifically for hospitals and healthcare institutions that produce hazardous biomedical waste. The Evacle 80 unit is a sterilizing shredder intended to treat pathogenic biological waste and convert it into a sanitary waste while simultaneously reducing the raw waste volume by up to 80% and turn it un-recognizable. On completion of the sterilization process, liquids are separated from solid waste, and the used, deactivated biocide is disposed into the drainage system. Solids are collected into a trash bin and discarded to the general waste.

Objectives

The objective of this efficacy test validation was to examine the claims presented by the manufacturer regarding the microbial inactivation efficacy of the Evacle 80 unit, accompanied by the sterilant EVACETIC. The manufacturer claims to comply with STAATT Level IV sterilization, which is defined as the reduction of mycobacteria viable cells by 6 $\log_{10}$ CFU/ml. The biological indicator was chosen by the lab as the STAATT guidelines accept all mycobacteria species.

References

- ISO 11737-1:2006(E) Sterilization of medical devices - Microbiological methods. Part 1: Determination of population of microorganisms on products
- Current USP, <61>, <62>, Microbiological Examination of Nonsterile Pharmaceutical Products
- Accredited Laboratory SOP 50.WI.106: Bioburden Test - Presterilization Estimation of population of microorganisms in/on Medical Device
- Other specific Accredited Laboratory SOPs

Materials and Methods

Reference Strains

Culture suspension prepared by Accredited Laboratory *Mycobacterium smegmatis* (ATCC 607). mycobacteria viable cells suspension in concentration of 2.82 x 10^7 CFU/ml.

Surrogate Waste Composition

The constructed to contain 10% w/w organic material and 90% of inorganic waste. The total weight of the sample was 160 gr. The simulated inorganic material consisted of clean medical waste components, such as metals and plastic, which represents the process worst case due to their structure or reaction with the Evacetic (90% of the total weight). The simulated organic material consisted of 16 gr (10% of the total weight) of blood agar.

Sterilizing Solution

Sterilization is performed by the Evacetic solution. The solution active substance is peracetic acid (PAA), accompanied by hydrogen peroxide. Both substances are strong oxidizers and act synergistically to create high sporicidal activity.

Laboratory Procedure

1. The sample of 160 gr representing shredded medical waste inoculated with 5 ml of *Mycobacterium* suspension containing 2.82 x 10^7 CFU/ml (total 1.4 x 10^8) and mixed.
2. The inoculated waste sample was suspended in 500 ml, 1% EVACETIC solution, and incubated for 12 min.
3. Following the incubation, 3 portions of 10 gr each were sampled for bioburden testing.
4. Each sample was suspended in saline with 0.1% Tween 80, mixed and shaken for 15 min and examined by using the membrane filtration technique.
5. A total of 3 samples in order to check and validate the consistency of the results.

Results

Sample Solid Phase	Inoculum Size	Sample 1	Sample 2)	Sample 3
CFU/10gr	$8.7x10^6$	<1	<1	<1
CFU/sample	$1.4x10^8$	<16	<16	<16
Log	8.15	1.2	1.2	1.2
Avg. Log	NA	1.2		
Log Reduction	NA	6.95		

Table 2 – Sterilization results of Mycobacteria viable cells following sterilization process with the Evacle 80 unit

Conclusions

Based on the information detailed above, as well as the data presented in Table 2, it can be concluded that the Evacle 80 unit sterilization process' accompanied by the use of the disinfectant Evacetic allows the reduction of 6 $\log_{10}$ CFU/ml of vegetative Mycobacterium cells. This reduction complies with STAATT Level 4 according to the State and Territorial Association on Alternative Treatment Technologies (STAATT) guidelines.

User Safety Validation

Method

Introduction

The Evacle 80 unit has been designed specifically for hospitals and healthcare institutions that produce hazardous biomedical waste. The Evacle 80 unit is a sterilizing shredder intended to treat pathogenic biological waste and convert it into a sanitary waste while simultaneously reducing the raw waste volume by up to 80% and turn it un-recognizable. On completion of the sterilization process, liquids are separated from solid waste, and the used, deactivated biocide is disposed into the drainage system. Solids are collected into a trash bin and discarded to the general waste.

Objectives

The objective of this user safety validation test was to examine the claims presented by the manufacturer regarding the microbial inactivation efficacy of the Evacle 80 unit, accompanied by the sterilant EVACETIC. The manufacturer claims to comply with STAATT Level IV sterilization of the Evacle 80 hopper, where the untriturated waste is loaded into the unit. STAATT Level IV is defined as the reduction of 6 $\log_{10}$ CFU/ml of bacterial spores of the biological indicator Geobacilus stearothermophilus. The biological indicator was chosen according to STAATT guidelines.

The procedure was repeated 3 times to allow replication of the test in order to verify and validate the consistency of the results.

References

- ISO 11737-1:2006(E) Sterilization of medical devices – Microbiological methods. Part 1: Determination of population of microorganisms on products
- Current USP, <61>, <62>, Microbiological Examination of Nonsterile Pharmaceutical Products
- Accredited Laboratory SOP 50.WI.106: Bioburden Test – Presterilization Estimation of population of microorganisms in/on Medical Device
- Other specific Accredited Laboratory SOPs

Materials and Methods

Reference Strains – Spore Suspension

Terragene Bionova® commercial spore suspension BT20S/9 *Geobacillus Stearothermophylus* ATCC 7953 in concentration of 1.2 x 10_9 CFU/ml (to be diluted to 10^6 CFU/ml as part of the process); Lot # BT20S9072021; Expiration Date: July 2021.

Sterilizing Solution

Sterilization is performed by the EVACETIC solution. The solution active substance is peracetic acid (PAA), accompanied by hydrogen peroxide. Both substances are strong oxidizers and act synergistically to create high sporicidal activity. The loading hopper is equipped with spray nozzles, which disinfect the hopper during each cycle, by spraying Evaceticover the entire hopper surceases, followed by water washing.

Hopper Disinfection Process

The disinfection of the hopper process is comprised of the following parameters: Disinfecting Solution (EVACETIC), Temperature (°C), Contact Time (minutes), and Sterilant Concentration (ppm).

After waste shredding time is finalized, 50 ml concentrated disinfecting solution (EVACETIC) is sprayed out of 6 spray nozzles located on the hopper walls. The concentrated disinfecting solution is suspended for about 12 min. Once the waste sterilization cycle is over and the outlet valve is opened, the hopper is washed with water to allow safe opening of the hopper for loading the next cycle.

The Evacle 80 hopper disinfection process is characterized by the following parameters' values:

- Sterilant Concentration: 900-1000 ppm PAA (2% from stock 4.5-5% solution)
- Contact Time: 12 minutes.
- Water Washing: 30 sec.

Test Procedure Description

Prior to User Safety Validation test initiation, the Evacle 80 unit was loaded with efficacy test validation, load of 8 kg of waste inoculated with 25 ml of *Geobacillus Stearothermophylus* ATCC 7953 spore suspension in a concentration of 1.2×10^9 CFU/ml. The load was shredded and treated. Following the cycle when the hopper was opened, it was sampled using a swab kit – for bioburden testing:

- Two (2) swabs for the upper inside of the hopper cover (1, 2)
- Three (3) swabs for the hopper chamber walls above the spray nozzles (3, 4, 5)
- Three (3) swabs for the hopper chamber walls below the spray nozzles (6, 7, 8)

Laboratory Procedure

Each swab was vortexed for 1 min, the liquid was filtered through 0.45µm membrane filter. The filter was washed with Saline 0.9% with 0.1% Tween 80 and transferred to TSA plate; the plates were incubated for at 55±2°C for 4 days

Results

The following incubation CFU from each sample were counted. No bacterial growth occurred at each of the replicate samples.

Experiment	Sample Description	Sample No.	CFU / Sample
1	Upper inside of the hopper cover	1	<1
		2	<1
	Hopper chamber walls above the spray nozzles	3	<1
		4	<1
		5	<1
	Hopper chamber walls below the spray nozzles	6	<1
		7	<1
		8	<1
2	Upper inside of the hopper cover	1	<1
		2	<1
	Hopper chamber walls above the spray nozzles	3	<1
		4	<1
		5	<1
	Hopper chamber walls below the spray nozzles	6	<1
		7	<1
		8	1
3	Upper inside of the hopper cover	1	2
		2	1
	Hopper chamber walls above the spray nozzles	3	<1
		4	<1
		5	2
	Hopper chamber walls below the spray nozzles	6	2
		7	9
		8	1

Table 3 – Hopper Sterilization results of *Geobacilus Stearothermophylus* spores following sterilization process with the Evacle 80 unit

Conclusions

Based on the information detailed above, as well as the data presented in Table 3, it can be concluded that the Evacle 80 unit sterilization process' accompanied by the use of the disinfectant Evacetic allows the reduction of 6 $\log_{10}$ CFU/ml of bacterial spores both for the solid and liquid waste phases. This reduction complies with STAATT Level 4 according to the State and Territorial Association on Alternate Treatment Technologies (STAATT) guidelines.

Liquid Drainage Composition Validation

Method

Introduction

The Evacle 80 unit has been designed specifically for hospitals and healthcare institutions that produce hazardous biomedical waste. On completion of the sterilization process, liquids are separated from solid waste. The used formula poses no risk to the environment and is disposed into the drainage system.

Objectives

The objective of this Liquid Drainage Composition Validation test was to examine the claims presented by the manufacturer regarding the liquid drainage composition of the Evacle 80 unit.

The procedure was repeated 3 times to allow replication of the test in order to verify and validate the consistency of the results.

Materials and Methods

Starting Materials

The liquids involved in the Evacle 80 unit disinfection process are the Evacetic solution and water.

The Evacetic solution active substance are peracetic acid (PAA) and hydrogen peroxide. Both substances are strong oxidizers, which may harm the environment if discarded to the drainage system.

Test Procedure Description

The simulated medical waste load was constructed to contain 10% w/w organic material and 90% of inorganic waste. The total weight of the sample was 8 kg.

8 kg of simulated medical waste was prepared. The waste was loaded into the Evacle 80 unit and an automatic sterilization cycle was initiated according to the validated parameters detailed above.

The sample was collected from the derange pipe outlet.

Laboratory Procedure Description

The sample was delivered to the laboratory to test its composition by chemical titration.

Results

Following chemical titration of the sample, the following components were identified and measured:

Experiment	Component	Result	Unit
1	Peracetic acid	0.115	%
	Hydrogen peroxide	0.19	%
	Acetic acid	0.056	%
	pH (as is)	4.1	
2	Peracetic acid	0.120	%
	Hydrogen peroxide	0.232	%
	Acetic acid	1.053	%
	pH (as is)	4.10	
3	Peracetic acid	0.175	%
	Hydrogen peroxide	0.269	%
	Acetic acid	1.066	%
	pH (as is)	4.00	

Table 4 – Liquid Drainage Composition Results

Conclusions

Based on the information detailed above, as well as the data presented in Table 4, it can be concluded that the Evacle 80 unit liquid drainage composition is safe to be discarded to the swage as declared by the manufacturer.

Appendices

Terragene Bionova ® Commercial Spore Suspension BT20S/9 Geobacillus Stearothermophylus ATCC 7953 Concentration Verification – Laboratory Report

Test sample:
Geobacillus stearothermophylus (ATCC 7953) spores suspension supplied by the sponsor in ampoule. Bionova. Lot# BT0S9072021. Expiration date: 07/2021

Test media and solution:

- **Tryptic Soy Agar (TSA)**
  Neogen. Lot: US111887. Laboratory Batch: 11/2. Laboratory Expiration date: 24.04.2020
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 26.05.2020

Test method:
Spores concentration was determined in duplicates using pour plate method on TSA medium.

Results:

Geobacillus stearothermophylus ATCC 7953
CFU/ml (average of duplicates)
1.51×10^9

Artificial Test Soil – Product Brochure

Brand Name of Product	Artificial Test Soil 2015 (ATS2015)
Generic Name of Product	Artificial Test Soil 2015 (ATS2015)
Product Code Number(s)	ATS2015-1ML, ATS2015-9ML, ATS2015-100ML, ATS2015-500ML, ATS2015-500ML-2, ATS2015-B-9ML, ATS2015-B-100ML, and ATS2015-B-500ML.
Purpose of Product	Hemoglobin, protein and carbohydrate based standardized test soil in proportion found on clinically used medical devices.
Range of Applications for Product	ATS2015 has been formulated for simulated use soiling of medical devices, including flexible endoscopes, for the purpose of conducting cleaning validations and cleaning verifications. The reconstituted ATS2015 test soil contains the following markers: protein, hemoglobin, carbohydrate, lipids and insoluble fibers. Bone can be added if requested.
Key Specifications of Product	• 1X Vial with dry test soil component. • Dry mixture includes purified bovine proteins (hemoglobin, albumin), physiological salt, mucin, xanthan gum, egg yolk, and cellulose. • 20% defibrinated sheep blood is added after the dry mixture is reconstituted. • Viscosity of reconstituted ATS2015 is ~ 9cP using a vibrational viscometer. • ASTM D3359-97 Standard adhesion testing shows < 8% soil removal of ATS2015 when dried onto a stainless-steel surface.

Shipping & Storage	
Shipping Conditions & Requirements	
Storage Conditions	• Store vials with dry test soil at room temperature. • Store vials reconstituted at 2°C- 5°C for up to 2 weeks. Keep away from light and heat.
Packaging Conditions	
Shelf Life	18 months: See imprint.

Instructions for Using Product	
Description of Use (s)	The reconstituted ATS2015 has excellent adhesive characteristics for flexible endoscope applications such as cleaning validation or harvesting validation studies.
Preparation for Use	
Diagrams (drawings, pictures)	
Steps for Use of Product	A. **Reconstitute the dry powder and homogenize as follows:** ATS2015 1mL: add 0.8 mL sterile water and vortex/shake for ~5 minutes ATS2015 9 mL: add 7.2 mL sterile water and vortex/shake for ~10 minutes ATS2015 100 mL: add 80 mL sterile water and vortex/shake for ~10 minutes ATS2015 250 mL: add 200 mL sterile water and vortex/shake for ~10 minutes ATS2015 500 mL: add 400 mL sterile water and vortex/shake for ~10 minutes After complete mixing, let the foam settle for ~20 minutes.

Laboratory Reports of Efficacy Test Validation and User Safety Validation by *Geobacilus stearothermophilus* Spores

First Test

Test samples:
Eight (8) swabs
Three (3) bottles of the liquid sample from experiment
Three (3) bottles of solid waste sample from experiment

Test microorganisms:
Geobacillus stearothermophilus (ATCC 7953) commercial spore suspension, Bionova, Lot# BT20S9072021, Expiration date: 07/2021

Test media and solutions:

- **Tryptic Soy Agar (TSA)**
  Neogen, Lot: US111667, Laboratory Batch: 11/2, Laboratory Expiration date: 24.04.2020
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 26.05.2020

Test procedure:

1. Each swab was vortexed for 1 minute, liquid was filtered through 0.45µm membrane filter.
2. Filter was washed with Saline 0.9% with 0.1% Tween 80 and transferred to TSA plate.
3. All plates were incubated at 55±2°C for 4 days
4. Each one of 3 liquid samples (each in volume of 1000ml) was filtered in 4 portions of 250 ml. The filters were washed with Saline 0.9% with 0.1% Tween 80 and placed onto TSA plates.
5. Portion of 10g from each one of 3 solid samples was weight and transferred to a sterile bag containing 200 ml of saline with 0.1% Tween 80.
6. Mix was shaken for 15 min at 200RPM and examined for bioburden using membrane filtration technique.
7. All filters were placed onto TSA (Tryptic Soy agar) plates. The plates were incubated at 55±2°C for 4 days.

Results:

Product ID	Sample #	CFU/1000ml	CFU/10g
Liquid	1	<1	N/A
	2	<1	
	3	<1	
Solid waste	1	N/A	<1
	2		<1
	3		<1

Results:

Swab #	Swabbing location	CFU/swab
1	Upper inside of the lid	<1
2	Upper inside of the lid	<1
3	Inlet chamber walls above the spray nozzles	<1
4	Inlet chamber walls above the spray nozzles	<1
5	Inlet chamber walls above the spray nozzles	<1
6	Inlet chamber walls below the spray nozzles	<1
7	Inlet chamber walls below the spray nozzles	<1
8	Inlet chamber walls below the spray nozzles	<1

Second Test

Test samples:
Eight (8) swabs
Three (3) bottles of the liquid sample from experiment
Three (3) bottles of solid waste sample from experiment

Test media and solutions:

- **Tryptic Soy Agar (TSA)**
  Neogen, Lot: US111887, Laboratory Batch: 1/1, Laboratory Expiration date: 26.05.2020
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 17.06.2020

Test microorganism: *Geobacillus stearothermophylus* (ATCC 7953)

Test procedure:

1. Each swab was vortexed for 1 minute, liquid was filtered through 0.45µm membrane filter.
2. Filter was washed with Saline 0.9% with 0.1% Tween 80 and transferred to TSA plate.
3. All plates were incubated at 55±1°C for 6 days
4. Each one of 3 liquid samples (each in volume of 1000ml) was filtered in 10 portions of 100 ml. The filters were washed with Saline 0.9% with 0.1% Tween 80 and placed onto TSA plates.
5. Portion of 10g from each one of 3 solid samples was weight and transferred to a sterile bag containing 200 ml of saline with 0.1% Tween 80.
6. Mix was shaken for 15 min at 200RPM and examined for bioburden using membrane filtration technique.
7. All filters were placed onto TSA (Tryptic Soy agar) plates. The plates were incubated at 55±1°C for 6 days.

Results:

Product ID	Sample #	CFU/1000ml	CFU/10g
Liquid	1	<1	N/A
	2	<1	
	3	<1	
Solid waste	1	N/A	<1
	2		<1
	3		<1

Results:

Swab #	Swabbing location	CFU/swab
1	Upper inside of the lid	<1
2	Upper inside of the lid	<1
3	Inlet chamber walls above the spray nozzles	<1
4	Inlet chamber walls above the spray nozzles	<1
5	Inlet chamber walls above the spray nozzles	<1
6	Inlet chamber walls below the spray nozzles	<1
7	Inlet chamber walls below the spray nozzles	<1
8	Inlet chamber walls below the spray nozzles	1

Third Test

Test samples:
Eight (8) swabs
Three (3) bottles of the liquid sample from experiment
Three (3) bottles of solid waste sample from experiment

Test media and solutions:

- **Tryptic Soy Agar (TSA)**
  Neogen, Lot: US111887, Laboratory Batch:2/2, Laboratory Expiration date: 26.06.2020
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 30.06.2020

Test microorganism: *Geobacillus stearothermophylus* (ATCC 7953)

Test procedure:

1. Each swab was vortexed for 1 minute, liquid was filtered through 0.45µm membrane filter.
2. Filter was washed with Saline 0.9% with 0.1% Tween 80 and transferred to TSA plate.
3. All plates were incubated at 55±1°C for 5 days
4. Each one of 3 liquid samples (each in volume of 1000ml) was filtered in 10 portions of 100 ml. The filters were washed with Saline 0.9% with 0.1% Tween 80 and placed onto TSA plates.
5. Portion of 10g from each one of 3 solid samples was weight and transferred to a sterile bag containing 200 ml of saline with 0.1% Tween 80.
6. Mix was shaken for 15 min at 200RPM and examined for bioburden using membrane filtration technique.
7. All filters were placed onto TSA (Tryptic Soy agar) plates. The plates were incubated at 55±1°C for 5 days.

Results:

Product ID	Sample #	CFU/1000ml	CFU/10g
Liquid	1	<1	N/A
	2	<1	
	3	<1	
Solid waste	1	N/A	<1
	2		<1
	3		<1

Results:

Swab #	Swabbing location	CFU/swab
1	Upper inside of the lid	2
2	Upper inside of the lid	1
3	Inlet chamber walls above the spray nozzles	<1
4	Inlet chamber walls above the spray nozzles	<1
5	Inlet chamber walls above the spray nozzles	2
6	Inlet chamber walls below the spray nozzles	2
7	Inlet chamber walls below the spray nozzles	9
8	Inlet chamber walls below the spray nozzles	1

Laboratory Report of Efficacy Test Validation *Mycobacteria* Suspension Solution

Test microorganisms:
Mycobacterium smegmatis (ATCC 607) containing 1.00×10^9 CFU/ml

Test media and solutions:

- **Tryptic Soy Agar (TSA)**
  Neogen, Lot: US111667, Laboratory Batch: 7/3, Laboratory Expiration date: 31.03.2020
- **Buffer Sodium Chloride** pH=7.0
  Laboratory Expiration date: 02.04.2020.
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 06.04.2020

Test procedure:

I. Test method Validation –Membrane Filtration Method

Note:A validation of the MF technique, used for Bioburden determination, was performed in order to verify that there are no residuals of the disinfection agent on the membrane, which may inhibit microorganism's growth. This test was performed before initiating the disinfection processes on the test sample.

1. Three (3) portions, each of 495ml water was heated to 40-42^{0}C.
2. 5ml were added to each water portion.
3. Each portion (total volume 500 ml) was filtered through 0.45µm membrane filter
4. The filters were washed each with 450ml of Saline 0.9% with 0.1% Tween 80
5. Additional 50ml portion of washing solution containing test-microorganism was filtered through the same membrane.
6. 50 ml of sterile saline with 0.1% Tween 80 were filtered through 0.45µm filter membranes and were used for initial inoculum control
7. All filters were placed onto TSA plates. The plates were incubated at 30-35^0 for 6 days.

Acceptance Criteria:

The challenged test product shall demonstrate a number of recovered CFU not less than 70% of that recovered from the inoculum control

Results:

Test Microorganisms	CFU/ plate (average of 3)		% recovery
	Control membranes	Experimental membranes	
Mycobacterium smegmatis (ATCC 607)	94	91	96.8

Conclusion: The test met the acceptance criteria

II **Disinfection efficacy**

Test microorganisms:

Mycobacterium smegmatis (ATCC 607) containing 2.82×10^7 CFU/ml

Test media and solutions:

- **Tryptic Soy Agar (TSA)**
  Neogen, Lot: US111667, Laboratory Batch: 9/2, Laboratory Expiration date: 22.04.2020
- **Buffer Sodium Chloride** pH=7.0
  Laboratory Expiration date: 13.04.2020.
- **Saline 0.9% with 0.1% Tween 80**
  Laboratory Expiration date: 21.04.2020
- **Middlebrook 7H11+OADC**
  Hy Labs, Lot: 1116663, Expiration date: 07.04.2020

Test procedure

1. The sample of 160g representing shredded surrogate medical waste was placed into a beaker
2. 5ml of *Micobacterium* suspension containing 2.82×10^7 CFU/ml (total $1.4x10^8$) were added and mixed with the sample.
3. 495ml of preheated water were added followed by vigorous agitation.
4. Then 5ml of were added and immediately after that start the clock for 12 min.
5. Three portions of the solid phase, each of 10g, were sampled and transferred into sterile bag with 200ml of saline with 0.1% Tween 80.
6. Mix was shaken for 15 min at 200RPM and examined for bioburden using membrane filtration technique.
7. All filters were washed according to the validation test procedure.

8. **Results:**

Mycobacterium smegmatis (ATCC 607)				
Sample solid phase	Inoculum size	Sample 1	Sample 2	Sample 3
CFU/10g	$8.7x10^6$	<1	<1	<1
CFU/sample	$1.4x10^8$	<16	<16	<16
Log	8.15	1.2	1.2	1.2
Average Log	N/A	1.2		
Log Reduction	N/A	6.95		

Laboratory Reports of Liquid Drainage Composition Validation

First Test

Certificate of Analysis EVACETIC		
Test Desc.	**Result**	**Units**
Hydrogen peroxide	0.19	%
Peracetic Acid	0.1154	%
Acetic Acid	0.056	%
pH (as is)	4.1	

Second Test

Certificate of Analysis EVACETIC		
Test Desc.	**Result**	**Units**
Hydrogen peroxide	0.232	%
Peracetic Acid	0.12	%
Acetic Acid	1.053	%
pH (as is)	4.10	

Third Test

Certificate of Analysis EVACETIC		
Test Desc.	**Result**	**Units**
Hydrogen peroxide	0.269	%
Peracetic Acid	0.175	%
Acetic Acid	1.066	%
pH (as is)	4.00	