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Specialists in Microbiology & Regulatory Affairs

TEST REPORT
BIOBURDEN REDUCTION CLEANING VALIDATION STUDY
BUXTON ORTHOPEDIC SURGICAL INSTRUMENTS

CLIENT: Buxton BioMedical, Inc.
15A Melanie Lane 7
East Hanover, NJ 07936

TEST #: 13-1526 BBM

DATE: 09/25/13

REPORT TO: Alice Schussler

TEST ARTICLES:

Buxton Orthopedic Surgical Instruments

1) Cat. #24-6362, Mod.Mini-Open Frame f/3rd arm
2) Cat. #843-5242, Punch, 200x4mm, 40°up, Ejec, TFP
3) Cat. #SE-3035, Revolver Endo Shaft, 300 x 3.5, 40° (Assembled)
4) Cat. #46-1110, Arthro Curette, 13cm, 10°, #1
5) Cat. #852-R3-3936, Rev2 Shaft Op3, 195 x 3.2, 40° (Assembled)
6) Cat. #54-3011, K-K Tendon Retrvr, flex, 2.0mm

Refer to Buxton Validation Protocol, Report #VP-020613 "Validation of Buxton Cleaning Instructions for Use"

PURPOSE OF THE STUDY:

To determine the efficacy of the specified cleaning procedure for the reusable instruments by a bioburden reduction method.

PROCEDURE:

Reference Mycoscience Protocol: "Cleaning Validation Study for Buxton Orthopedic Surgical Instruments Bioburden Reduction Method, Protocol Rev. 09/11/13"

1.0 *Inoculation:*

Each instrument was inoculated with a suspension containing a minimum of 1.0×10^5 CFU/mL of *Geobacillus stearothermophilus* in an organic load containing 5% defibrinated sheep blood and 5% fetal bovine serum and 0.5% pig mucin. This organic load is based on the 3 protein soil recommended in AAMI TIR 30. The instruments were inoculated by directly immersing the devices. The mechanisms were actuated 3X to insure penetration of the soil to all areas, including those areas that would be considered hardest to clean. The inoculum was allowed to set on the devices in a laminar flow hood at room temperature and humidity for 30 minutes.

2.0 *Cleaning:*

See attached Buxton Validation Protocol Report #VP-020613 for cleaning instructions. The procedure includes soaking, thorough manual cleaning and rinsing. Enzol enzymatic cleaning solution was used for the cleaning cycles, prepared per the manufacturer's instructions. The solution was prepared using warm (37 – 40°C) water. The soak time used was 15 minutes.

3.0 *Bioburden Enumeration (Post Cleaning):*

The cleaned instruments were placed in individual sterile stomacher bags with enough sterile USP Fluid D to completely immerse the devices. The bags containing the submerged instruments were sonicated for 10 minutes. After sonication the bags were gently shaken to evenly distribute any residual microorganisms. Appropriate dilution volumes were removed and filtered through 0.45um bacterial retentive membrane filters. The filters were plated to Trypticase Soy Agar (TSA) plates and were incubated for 48-72 hours @ 55-60°C. The number of recovered *Geobacillus stearothermophilus* CFU/plate, was counted and multiplied by the dilution factor to obtain the total recovered CFU count.

4.0 *Positive Control Cycle:*

For the positive control baseline cycle, each instrument was inoculated as in Step 1.0. However, the cleaning procedure was not performed on these instruments. The bioburden enumeration in Section 3.0 was performed to determine initial inoculum levels on the instruments. The results from this cycle were compared to the results of the cleaning cycles and provided the baseline for the mean log reduction determination for these instruments.

RESULTS:

BUXTON BIOMEDICAL ORTHOPEDIC SURGICAL INSTRUMENTS

POST CLEANING BIOBURDEN RECOVERY

Instrument	Post-Cleaning CFU RECOVERED			
	Cycle #1	Cycle #2	Cycle #3	Mean
1) Cat. #24-6362, Mod. Mini-Open Frame f/3rd arm	154	58	64	92
2) Cat. #843-5242, Punch, 200x4mm, 40°up, Ejec, TFP	140	128	130	133
3) Cat. #SE-3035, Revolver Endo Shaft, 300 x 3.5, 40° (Assembled)	40	<2	24	22
4) Cat. #46-1110, Arthro Curette, 13cm, 10°, #1	36	2	86	42
5) Cat. #852-R3-3936, Rev2 Shaft Op3, 195 x 3.2, 40° (Assembled)	228	124	128	160
6) Cat. #54-3011, K-K Tendon Retrvr, flex, 2.0mm	72	14	108	65

LOG REDUCTION OVER UNCLEANED POSITIVE CONTROL

Instrument	Positive Control CFU Recovered	Control Log_{10}	Mean CFU Recovered	Log_{10}	Microorganism Log Reduction
1) Cat. #24-6362, Mod. Mini-Open Frame f/3rd arm	1.9×10^5	5.28	92	1.96	3.32
2) Cat. #843-5242, Punch, 200x4mm, 40°up, Ejec, TFP	1.6×10^5	5.20	133	2.12	3.08
3) Cat. #SE-3035, Revolver Endo Shaft, 300 x 3.5, 40° (Assembled)	7.9×10^4	4.89	22	1.34	3.55
4) Cat. #46-1110, Arthro Curette, 13cm, 10°, #1	1.72×10^5	5.24	42	1.62	3.62
5) Cat. #852-R3-3936, Rev2 Shaft Op3, 195 x 3.2, 40° (Assembled)	2.7×10^5	5.43	160	2.20	3.23
6) Cat. #54-3011, K-K Tendon Retrvr, flex, 2.0mm	8.9×10^4	4.95	65	1.81	3.14

Test Start Date: 9/18/13 **Test Completion Date: 9/25/13**

CONCLUSION:

When tested as described, the attached cleaning instructions met or exceeded the acceptance criteria established of a minimum bioburden log reduction of 2.0, and no visible soil remaining on the cleaned instruments. The cleaning instructions demonstrated a mean microorganism log reduction range of 3.08 - 3.62 and the instruments had no visible soil remaining after cleaning. Under the conditions of the test, the described manual cleaning procedure for the Buxton Orthopedic Surgical Instruments was determined to be effective.

TEST REFERENCE:

1. AAMI TIR12:2010, *Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Healthcare Facilities: A Guide For Medical Device Manufacturers.*
2. AAMI TIR30:2011, *A Compendium of Processes, Materials, Test Methods, and Acceptance Criteria for Cleaning Reusable Medical Devices*

Analyst: Amy Cameron Date: 9/25/13

Reviewed by: R. Arsenault Date: 9/25/13

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CLEANING VALIDATION STUDY FOR BUXTON ORTHOPEDIC SURGICAL INSTRUMENTS BIOBURDEN REDUCTION METHOD PROTOCOL REV. 09/11/13

Sponsor

Buxton BioMedical, Inc.
15A Melanie Lane 7
East Hanover, NJ 07936

Test Facility:

Mycoscience Labs
25 Village Hill Rd.
Willington, CT 06279

Purpose of the Study:

To determine the efficacy of the specified cleaning procedure for the reusable instruments by a bioburden reduction method.

Test System and Justification:

This study will evaluate the sterilization efficacy of the devices in conformance with the AAMI TIR # 12-2010: *Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A Guide For Medical Device Manufacturers* and AAMI TIR #30-2011: *A Compendium of Processes, Materials, Test Methods and Acceptance Criteria for Cleaning Reusable Medical Devices.*

Test Articles:

Buxton Orthopedic Surgical Instruments

1) Cat. #24-6362, Mod.Mini-Open Frame f/3rd arm
2) Cat. #843-5242, Punch, 200x4mm,40°up,Ejec,TFP
3) Cat. #SE-3035, Revolver Endo Shaft, 300 x 3.5, 40° (Assembled)
4) Cat. #46-1110, Arthro Curette, 13cm, 10°, #1
5) Cat. #852-R3-3936, Rev2 Shaft Op3, 195 x 3.2, 40° (Assembled)
6) Cat. #54-3011, K-K Tendon Retrvr,flex,2.0mm

Refer to Buxton Validation Protocol, Report #VP-020613 "Validation of Buxton Cleaning "Instructions for Use"

Control Articles:

The test articles above will be used as a positive (baseline) control sample.

Methods:

1.0 Inoculation and Cleaning Procedure

1.1 Inoculation and Verification (Bioburden) Procedure

1.1.1 The instruments will be inoculated with a minimum concentration of $1.0x10^5$ *Geobacillus stearothermophilus* in an organic load of 5% bovine serum, 5% defibrinated sheep blood, and 0.5% porcine mucin in sterile phosphate buffered water. **This is a 3-protein soil recommended for**

testing surgical instruments in AAMI TIR 30. Inoculate the instruments by direct immersion in the organic soil and actuate any mechanisms on the instruments to three times insure penetration of the soil to all areas, including those areas that would be considered hardest to clean. Allow the inoculum to set on the instruments in a laminar flow hood for 30 minutes.

1.1.2 Run a positive control sample (not cleaned) for each instrument. Inoculate the positive control as above. Verify the inoculum density on the control instruments by using enough sterile USP Fluid D in a stomacher bag to immerse the instrument. Sonicate each instrument for 10 minutes. Note the total volume of Fluid D in order to calculate serial dilutions.

1.1.3 After sonication, gently shake the bag to evenly distribute residual microorganisms, then filter suitable volumes of each extract through 0.45um membrane filters to obtain a readable plate count. Place the membranes onto the surface of Trypticase Soy Agar (TSA) plates and incubate at 55-60°C for 48 hours.

1.1.4 Record the recovered microorganism count from each positive control test instrument. This number will serve as the baseline value for calculating the percent reduction of the inoculum after performing the cleaning procedure.

1.2 Cleaning Procedure

1.2.1 Clean the test articles **per Buxton Validation Protocol, Report #VP-020613 cleaning instructions**.

1.2.2 After cleaning the instruments, place them in individual sterile stomacher bags and add enough sterile USP Fluid D to immerse all the inoculation points. Sonicate each instrument for 10 minutes.

1.2.3 Perform the bioburden testing described in section 1.1.3 -1.1.4

1.2.4 Clean and dry the instruments thoroughly, then repeat the inoculation, cleaning, and bioburden testing until three cleaning cycles on the instruments have been completed.

2.0 Calculation of Efficacy

2.1 Calculate the percent bioburden reduction and log reduction for each of the cleaning cycles by comparing to the uncleaned control instrument.

2.2 Acceptance criteria:

1) No visible soil on the cleaned instruments.
2) Minimum 2 Log reduction in the inoculated bioburden after the cleaning process.

The results will include all pertinent recovery and bioburden data.

Report:

The final report will include an identification of all the test articles, a summary of the methods used, results, deviations, and any other pertinent information.

References:

1) AAMI TIR #12-2010: Designing, Testing, Labeling Reusable Medical Devices For Reprocessing in Healthcare Facilities: A Guide For Device Manufacturers.
2) AAMI TIR #30-2011: *A Compendium of Processes, Materials, Test Methods and Acceptance Criteria for Cleaning Reusable Medical Devices.*

Approvals:

Mycoscience Representative — Date: 18 Sept. 2013

Buxton BioMedical Representative — Date: 18 Sept 2013

VALIDATION PROTOCOL

Date: February 6, 2013 REPORT #: VP 020613

To: **FILE** Page 1 of 3

From: Ed Schussler

Subject: **VALIDATION OF BUXTON CLEANING "*INSTRUCTIONS FOR USE*"**

Ref.: AAMI TIR No. 12-1994 Design, Testing and labeling Reusable Medical Devices for Reprocessing in Health Care Facilities:: A Guide for Device Manufacturers
ANSI/AAMI ST79 *Comprehensive Guide to Steam Sterilization & Sterility Assurance in Healthcare* Facilities
HTM 2010 Health Technical Memorandum for Sterilization in the United Kingdom

OBJECTIVE: The objective of this protocol is to provide evidence that recommended instrument reprocessing cleaning method presented in Buxton's Cleaning Certification form and package insert *("Instruments that Make Music In Surgery" red, tan & brown card)* are adequate to ensure appropriate bioburden reduction.

SCOPE: The scope of this study was to verify the effectiveness of the cleaning methods presented in Buxton's Clean Certificate Form and package inserts. The methods recommend cleaning as:

- Inspect this instrument upon receipt and carefully clean before sterilizing in preparation for its initial use.
- Clean and disinfect this instrument immediately after each use. Instruments should be wiped clean of gross debris and biofluids and kept moist until they reach sterile processing. Rinse thoroughly with lukewarm water.
- Scrub with soft brushes and, where necessary, flush out crevice joints and small orifices with a syringe filled with demineralized water. Use low-foaming, enzymatic cleaning solutions with a pH range of 6-8, and follow the manufacturer's instructions.
- Rinse with demineralized water. Lubricate with a water-soluble instrument milk and dry completely before storing.

Since Buxton has over 5,600 surgical instruments in their product-line, it is impossible to validate a cleaning instruction for each instrument. Based on this, with the help of a medical device/pharmacological microbiologist specialist, Buxton chose 6 instruments that could be considered "worst case" / "most difficult" to clean based on complexity, narrow lumens, multiple mated surfaces and interfaces that could potentially hide debris.

MATERIALS:

Geobacillus Stearothermophilus Suspension >10^5 CFU/mL
Organic Load (5% defibrinated sheep blood & 5% fetal bovine serum)
Micro-pipetto
Laminar Flow Bench / Hood
Comtrex Enzymatic Detergent Solution
Heated Bath - Water / Enzymatic Solution (1% concentration) (~37-40°C)
Soft bristle brushes
Tubular soft bristle brushes
Distilled water
60cc syringe with Luer hub
NAMSA SUS-06 *(Source of 1.0x106 spores of Bacillus stearothermophilus)*
Stomacher Bag w/ 500ml sterile fluid D
Ultrasonic Bath
0.45micro filter
Petri dishes – Trypticase Soy Agar
Incubator 55-60°C
(16) "Worst case" Representative Buxton Surgical Instruments (See Family Tables)
Buxton Shoulder Surgery Set including Metal Case (See Family Table)

Family #1: Complete Hinged / Mated Surfaces

Sample	Catalog #	Instrument Family
1.1	24-6362	Mod.Mini-Open Frame f/3rd arm

Family #2: Sliding & Long Rubbing Parts

Sample	Catalog #	Instrument Family
2.1	843-5242	Punch, 200x4mm,40°up,Ejec,TFP

Family #3: Rough Surfaces & Cannulated

Sample	Catalog #	Instrument Family
3.1	SE-3035	Revolver Endo Shaft, 300 x 3.5, 40°
3.2	46-1110	Arthro Curette, 13 cm, 10°, #1
3.3	852-R3-3936	Rev2 Shaft Op3, 195 x 3.2, 40°

Family #4: Flexible Instruments (Coiled Shafts & Metal Mesh)

Sample	Catalog #	Instrument Family
4.1	54-3011	K-K Tendon Retrvr,flex,2.0mm

ATTACHMENTS:

Attachment A – Buxton Cleaning Certificate (Form SOP-751-002-1, Rev2)
Attachment B - Buxton Package Insert "Instruments that Make Music In Surgery!" (red, tan & brown card)
Attachment C – Buxton Product Insert "EndoShaft Instructions"

PROCEDURE:

INOCULATION PHASE: Inoculate each instrument with a suspension of > >10^5 CFU/mL Geobacillus Stearothermophilus in an organic load. Inoculate the instruments with a mico-pipettor and evenly distribute 50% of the inoculums to the instrument exterior and 50% to the inner lumens, mated surfaces or interfaces as appropriate to the device. Allow inoculated devices to dry in laminar flow hood for one hour.

CLEANING PHASE: Place inoculated instruments into warm (37-40 °C) bath containing 1% concentration of enzymatic cleaning solution. Remove any visible soil with as soft bristle brush and flush any lumens with the enzymatic cleaning solution using a 60cc syringe. Allow all instruments to soak for minimum of 15 minutes in the warm detergent batch. After 15 minutes, scrub instrument exterior with a soft bristle brush. Using an appropriate size cleaning brush to clean any lumens or crevices. After brushing, thoroughly rinse each instrument in its entirety using DI water. Use as syringe or hose attached to the DI water to thoroughly flush all lumens. Dry instruments with clean compressed air at room temperature.

The inoculation and cleaning process should be performed three times for each instrument. In addition, a base line positive control for each device should be inoculated as above but, not subjected to the described cleaning process.

BIOBURDEN ENUMERATION PHASE: Place the cleaned and rinsed instruments into a sterile stomacher bags with 500mL of Fluid D. Each family of instruments should be tested as a composite sample, with 4 devices per stomacher bag. The bag should be placed in an ultrasonic bath and sonicate for 10 minutes. Manually shake the bags gently for 15 seconds immediately prior to sampling. Remove appropriate dilution volumes and filter through a 0.45µ membrane filter. The filter membrane should be plated into a Petri of Trypticase Soy Agar and incubated at 55-60°C for 2-3days and enumerated.

CYCLES REQUIRED FOR STUDY COMPLETION: The inoculation and cleaning process should be performed three times for each instrument.

POSITIVE CONTROL: For the positive control baseline cycle, the (5) instrument families are inoculated as outlined. However, the cleaning procedure is not performed on each set of instruments. Proceed directly to the bioburden enumeration phase of this outline. The results from this cycle are compared to the mean results of (3) cleaning cycles and will provide a basis for the mean log reduction.

INTERPRETATION:

A successful (effective cleaning methodology) validation study will require:

1) No visible soil be found on the cleaned devices
2) Significant (at least a 2-log) reduction in the inoculated bioburden after the cleaning process.