Arthrex, Inc.
Study Protocol:

Sterility Validation of Arthroscopy Pump Tubing with Reusable Main / Disposable Extension.

Written by: Sean M. Colwell (Bec Laboratories, Inc.) Date: 5-20-98
Title: Asst. Manager

Approved by: Scott M Dunlachen (Arthrex, Inc.) Date: 5/26/98
Title: Director of RA/QA

Biological & Environmental Control Laboratories, Inc.
705 Front St.
Toledo, OH 43605

1.0 PURPOSE:

The purpose of this study is to determine the ability of the Arthrex, Inc. Arthroscopy Extension Tubing Set (part # AR-6220 - hereafter referred to as an Extension Set) check valve to prevent retrograde contamination of a single Pump Tubing Set (part # AR-6210 - hereafter referred to as a Main Set) after use of multiple Extension Sets or repeated use of a single Extension Set.

2.0 APPLICABLE DOCUMENTS

AAMI TIR No. 12 - 1994. Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A guide for Device Manufacturers. (applicability to the concentration of challenge suspension and other properties of reusable devices)

3.0 MATERIALS AND EQUIPMENT

3.1 Test Organisms:

3.1.1 *Bacillus subtilis* var *niger* at 10^3 colony forming units (CFU's) per ml
3.1.2 *Bacillus subtilis* var *niger* at less than 100 CFU's per inoculum

3.2 Solutions and Media

3.2.1 0.9% Sodium Chloride, USP, 1L
3.2.2 Phosphate buffer, sterile
3.2.3 Tryptic Soy Broth (TSB), 30 ml, sterile
3.2.4 Tryptic Soy Broth (TSB), 100 ml in screw cap closure, sterile
3.2.5 Deionized H_2O, sterile
3.2.6 Tryptic Soy Agar (TSA), 100 ml in screw cap closures, sterile
3.2.7 Disinfectant Solution (theoretical 200 ppm hypochlorite solution)

3.3 Test Equipment

3.3.1 Incubator, 30-35° C
3.3.2 Analytical Filter Unit, 0.45 µm, sterile
3.3.3 Filter Flask, 1000 ml
3.3.4 Vacuum source
3.3.5 Class 100 Laminar Air Flow Hood
3.3.6 Forceps
3.3.7 Micropipetter, 20 - 200 µl; Pipette Tips, sterile
3.3.8 1 cc Syringe, sterile; 21 1/2 gauge needle, sterile
3.3.9 Centrifuge, refrigerated
3.3.10 Centrifuge tubes, 50 ml, sterile
3.3.11 Peristaltic Pump (see Addendum I)
3.3.12 Nitrogen compressed gas cylinder (reference Addendum I)
3.3.13 Pressure regulator, accurate at 4.8 p.s.i. (reference Addendum I)
3.3.14 Pressure Vessel (reference Addendum I)

4.0 PROCEDURES

4.1 Preparation of suspensions

4.1.1 Perform a population verification on a stock suspension of *B. subtilis* var *niger* by serially diluting the suspension in 9.0 ml sterile deionized water blanks. Plate to sterile petri dishes in duplicate 1.0 ml of each 10^{-6} through 10^{-9} dilutions and incorporate into molten and tempered TSA. Incubate plates inverted at 30-35 ° C for 48 hours.

4.1.2 Remove plates from incubation and enumerate. Prepare the challenge suspension by adjust the population to 10^3 CFU's per ml to a volume of 1 liter using the following formula:

C1V1 = C2V2 where:
C1= concentration of the stock suspension
V1= volume of stock to be added to working suspension
C2= concentration of the working suspension (10^3 CFU's/ml)
V2= volume of working suspension (1 liter)

Verify population of the challenge suspension as outlined in 4.1.1. and transfere the challenge suspension to the pressure vessel.

4.1.3 Using the stock suspension in 4.1.1, adjust the population to less than 100 CFU's per 0.1 ml. This suspension is for the bacteriostasis / fungistasis test. Verify population as outlined in section 4.4.3.

4.2 Challenge of the Main Set with single and multiple Extension Sets.

4.2.1 Disinfect the interior surfaces of a Class 100 laminar air flow hood with an appropriate concentration of disinfectant solution.

4.2.2 Disinfect the exterior of the pump, Main Set packaging, Extension Set package, pressure vessel containing challenge suspension, two sterile 1 liter receiving jars, 14 sterile small jars, and the 0.9% sodium chloride solution bags. Place each unit into the laminar flow hood immediately after disinfection (don sterile gloves for all testing performed in this protocol and change gloves between each Extension Set test).

4.2.3 Aseptically open and connect the Main Set to the peristaltic pump and prime with the sterile 0.9% sodium chloride. Allow a small portion of the saline to flow into the sterile 1 liter receiving jar.

4.2.4 Aseptically open and attach an Extension Set to the Main set.

4.2.5 Turn paristaltic pump on to allow saline to flow through the Extension Set into the sterile receptacle and turn off pump. Once the Extension Set is primed, transfer the distal end to the pressure vessel containing the *B. subtilis* var *niger* solution and immerse in the challenge solution (reference Addendum I).

4.2.6 Using a pressure regulator attached to a cylinder of compressed nitrogen, apply approximately 250 mm Hg (4.8 p.s.i.) pressure to the pressure vessel for ten minutes (reference Addendum I).

4.2.7 Relieve the pressure and remove the Extension Set from the sealed pressure vessel. Disconnect the Extension Set from the Main Set and transfer the distal end of the Main Set to a sterile receiving jar (small jar) that is opened and presented by a second technician using aseptic process. Turn the paristaltic pump on and allow approximately 100 ml of saline to flow through the end of the Main Set into the sterile receptacle. The second technician closes the receptacle and labels as ETS #1 (Extension Tubing Set #1).

4.2.8 Repeat steps 4.2.4 through 4.2.7 until five different Extension Sets have been challenged and the effluents collected. The collected effluents from samples 2 through 5 should be labeled as ETS #2 - ETS #5.

4.2.9 Aseptically replace the Main Set with a new sterile unit. Follow steps 4.2.4 through 4.2.7 a total of five times using the same Extension Set for each challenge. The effluents from each of these tests should be clearly labeled as ETS #6A - ETS #6E.

4.3 Sterility Testing of the Main Set Effluent

4.3.1 Transfer all effluent from step 4.2.7 to the Sterility Testing Suite and test per BEC Standard Operating Procedure MST-26 (current revision): ~~Procedure for USP Sterility Test - Membrane Filtration~~ (reference attachment). Procedure for inoculated products as detailed in SOP MDT-26 (current revision) section 4.2.5 should be followed for the purposes of this validation. The TSB containing the filters should be incubated at 30 -35 °C instead of the 20 -25 ° C specified in BEC SOP MDT-26 (current revision) section 4.3.2.

4.4 Bacteriostasis / Fungistasis Testing

4.4.1 Perform Steps 4.2.1 through 4.2.5; Do not transfer distal end of Extension Set to the challenge suspension.

4.4.2 Using a sterile syringe and needle, aseptically inject 0.1 ml of the less than 100 cfu / 0.1 ml suspension into the Main Set approximately 5 cm from the Extension Set junction (reference Addendum II)

4.4.3 Transfer the distal end of the Extension Set to the pressure vessel containing the challenge suspension and continue to process the inoculated Main Set as per 4.2.6 through 4.2.7 Perform the sterility test as outlined in BEC SOP MDT-26 (current revision).

4.4.4 Repeat 4.4.1 through 4.4.3 using new Main and Extension Sets.

4.4.5 Using the same syringe and needle from 4.4.2, transfer duplicate 0.1 ml aliquots to separate sterile petri dishes and incorporate with 20 - 25 ml molten and tempered TSA. Allow plates to harden and incubate inverted at 30 - 35° C for 48 hours. This is the inoculum verification

4.4.6 Inoculate duplicate 100 ml SCD in the same manner as outlined in 4.4.2 as a control for the growth conditions. Incubate controls at 30 - 35 °C for seven days or until positive.

4.4.7 Results of the Bacteriostasis / Fungistasis Test should be interpreted as outlined in BEC SOP MST-14 (reference attached). All media for Bacteriostasis / Fungistasis testing should be incubated as specified in 4.3.1 of this protocol. Inoculum Verification should be less than 100 CFU's per dose.

4.5 Positive Control

4.5.1 Perform steps 4.2.3 through 4.2.6 in duplicate using separate Main Set and Extension Sets for each challenge. Removing the extension Set and place the end of the Main Set directly into the challenge suspension and immerse for 30 seconds.

4.5.2 Continue testing as per steps 4.2.7. Label the receptacle containers for the two samples tested in 4.5.1 as "positive controls" and process as specified in 4.3.1.

5.0 REPORTING

The final report of this protocol will be issued by BEC Laboratories, Inc. The report will include a final copy of the protocol as well as all sterility and bacteriostasis / fungistasis test results and any deviations from this initial protocol.

Naples, FL 34104
ATTN: Scott Durlacher

705 FRONT STREET • TOLEDO, OHIO 43605
PHONE: (419) 693-5307 • FAX: (419) 691-0418

lab no. 98T05055
p.o. no. 10935

rev: 0

SAMPLE
DESCRIPTION: Arthroscopy Pump Tubing - Sterility Validation of Resuable Main/Disposable Extension

TEST DATE: 05/22/98
RELEASE DATE: 05/29/98

ANALYSIS: Sterility Test

PROCEDURE: For complete procedure reference the Study Protocol and Product Test Procedur MST-26 Standard Operating Procedures for B.E.C. Laboratories, Inc.

RESULTS:	# Positive	# Negative
10 filters tested in 100 ml of SCD	0	10

CONTROL:

2 filters tested in 100 ml of SCD	2	0

CONCLUSION: Samples as submitted found to be STERILE when tested in accordance with the referenced Product Test Procedure and Guidelines from current USP.

date completed: 5/29/98 | tech: SL/SC | approved by:

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ATTN: Scott Durlacher

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lab no. 98T05056
p.o. no. 10935

rev: 0

SAMPLE DESCRIPTION: Arthroscopy Pump Tubing - Sterility Validation of Resuable Main/Disposable Extension

ANALYSIS: Preparation of the Challenge Suspension - *Bacillus subtilis* var *niger* @ 1 x 10^3 CFU's/ml

PROCEDURE: The challenge suspension of *Bacillus subtilis* var *niger* was prepared as outlined in the Sterility Validation of Arthroscopy Pump Tubing with Reusable Main/Disposable Extension study protocol section 4.1-Preparation of Suspensions. The population of the challenge suspension was verified at 1.4 x 10^3 CFU's/ml on the day of the challenge.

date completed:	tech:	approved by:
05/29/98	SC	Sean N. Colwell

Naples, FL 34104
ATTN: Scott Durlacher

B.E.C. Laboratories, Inc.
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PHONE: (419) 693-5307 • FAX: (419) 691-0418

lab no. 98T05061
p.o. no. 10935

rev: 0

SAMPLE DESCRIPTION: Arthroscopy Pump Tubing - Sterility Validation of Resuable Main/Disposable Extension

ANALYSIS: Bacteriostasis/Fungistasis

PROCEDURE: For complete procedure reference the study protocol and MST-14 Procedure for Bacteriostasis and Fungistasis Testing for B.E.C. Laboratories, Inc. Product samples were prepared as described in Study Protocol.

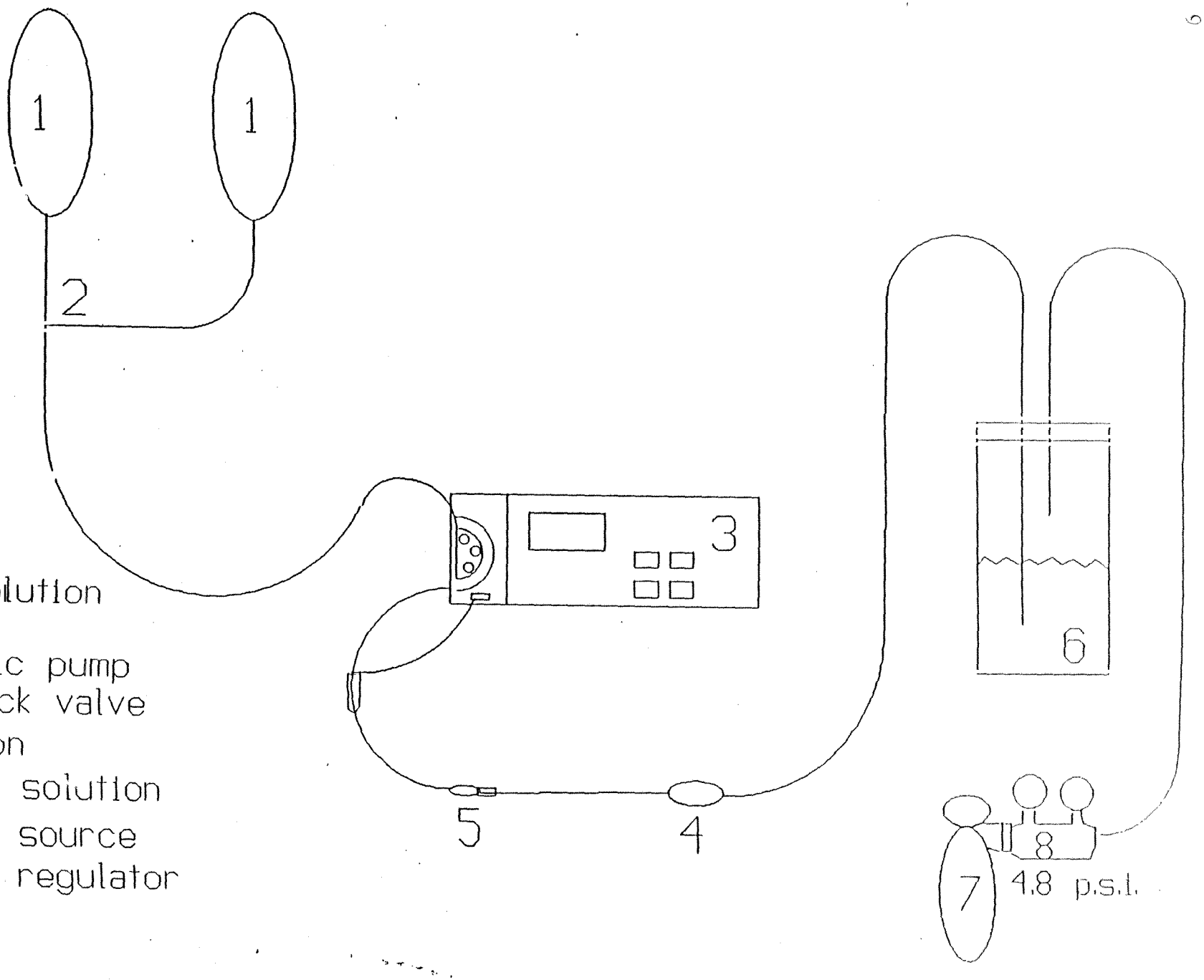
RESULTS: Medium: SCD Lot # 040198

Organism	Mean Inoculum Count	Duplicate 100 ml/Test	Duplicate 100 ml/Control
B. subtilis var *niger*	63.5	+ @ 4 days + @ 4 days	+ @ 4 days + @ 4 days

CONCLUSION: No bacteriostatic or fungistatic properties were associated with this product tested in 100 ml of SCD.

date completed:	tech:	approved by:
05/26/98	SC	Jean M. Colwell

Addendum I



1 Saline solution
2 Main Set
3 Peristaltic pump
4 Back check valve
5 Connection
6 Challenge solution
7 Pressure source
8 Pressure regulator

AR-6220 EXTENSION TUBING

PREPARATION FOR USE

1. The AR-6220 Extension Tubing is to be used in conjunction with the Arthrex Main Pump Tubing. The Extension Tubing Set is to be replaced at each subsequent arthroscopic procedure.

2. Prepare the arthroscope or the cannula for use with the tubing set.

3. Using sterile technique, carefully remove the tubing set from the package and pass it onto the sterile field.

4. Connect the Extension Tubing Set to the Pump Tubing Set at the luer fittings.

5. Release the tube clamp from the pump tubing and bleed the entire tubing system to eliminate any air pockets or bubbles in the tubing.

6. Connect the patient end of the tubing set to the arthroscope or cannula to begin irrigation of the joint.

STERILIZATION:
The tubing set is a single use item and is shipped sterile. Do not resterilize or reuse.

STORAGE:
Store in a cool dry place.

CAUTION:
Federal law (USA) restricts this device to sale by or on the order of a physician.

Arthrex.
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DFU-0003
Rev. 5

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PREPARATION FOR USE

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DFU-0003
Rev. 5

Arthrex®

DATE: 5/25/99

TO: ALL ARTHREX DISTRIBUTORS AND AGENTS

FROM: PHILIP S. O'QUINN

RE: REDEUCE TUBING SYSTEM

Previously, we contracted an independent testing lab to validate the fluid sterility when using our AR-6220 Extension Tubing as intended. The results proved our position that fluid sterility is maintained when using this product properly. For more information, refer to the memo dated 6/12/98, RE: AR-6220 Extension Tubing Sterility Validation Test Results, and the Test Protocol and Results from BEC Laboratories, Inc. dated 05/26/98.

These results are also applicable to the Patient ReDeuce Tubing (AR-6421). The materials used to construct both the AR-6220 and the AR-6421 are identical. The only component change between the two sets is the substitution of a luer fitting with a high-flow, shielded connector. The functional use of the product and the manner in which it is handled by the OR staff has not changed.

Arthrex Inc. • 3885 Seaboard Commerce Drive • Naples, Florida 34104 • Tel: 800 933-7601 or (941) 643-5553 • Fax: (941) 643-6218 • Website: www.arthrex.com