

BROOKHAVEN NATIONAL LABORATORY CLINICAL RESEARCH CENTER POLICY	NUMBER: IC-09	PAGE 1 OF 2
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SUBJECT: Sterility & Pyrogen Testing	REVIEWED BY: J. Rowan	CRC Manager
	APPROVED BY: H. Benveniste	Medical Dept. Chair
	EFFECTIVE DATE:	
	REVISION HISTORY:	

1.0 PURPOSE AND SCOPE

This document defines the Dept. policies and procedures concerning sterility testing and pyrogen testing. The requirements in it are intended to comply with FDA regulations regarding testing of solutions to be administered to research subjects for sterility and/or the presence of pyrogens.

2.0 POLICIES & PROCEDURES

2.1 Sterility Testing

The following is the procedure to be followed for testing done on-site. Testing for each batch of test solution by independent vendors may be an acceptable alternative (see Attachment 1).

Equipment:

1. Laminar flow hood (certified per JCAHO Rules and Regulations).
2. Refrigerator (temperature monitored and recorded using NIST thermometer by authorized designee)
3. Freezer: (temperature monitored and recorded using NIST thermometer by authorized designee)
4. Incubator: (temperature monitored and recorded using NIST thermometer by authorized designee)

Materials:

1. Tryptic Soy Broth (TSB) tubes: Remel, Lenexa, KS
2. Thioglycolate medium tubes: Remel, Lenexa KS
3. Blood agar plates: Remel, Lenexa, KS
4. Anaerobic bags: Remel, Lenexa KS
5. Microorganisms: Bacteroides vulgatus, Candida albicans, Bacillus subtilis: ATCC, Rockville, MD
6. Bacterial Transfer loop
7. Pyrogen free pipettes: Costar, Cambridge, MA
8. Sterile Saline: Abbott Laboratories, Chicago, IL

NOTE: All materials used must be within the expiration date. Alternative suppliers may be considered.

Procedures:

1. All procedures shall be carried out inside a certified laminar flow hood following the requirements of the Dept. Guideline QAU-2.9.0, "Laminar Flow Hoods". All materials coming in contact with the test solution must be sterile.
2. All bacterial cultures are tested before each subject study by streaking on to blood agar plates. A single colony from each plate is transferred to the appropriate broth growth medium. B. vulgatus is grown anaerobically, B. subtilis and C. albicans are grown aerobically.
3.
 - a. The test solution is withdrawn from a sterile bottle aseptically with a syringe and 0.1 ml each is placed into four sterile 18 ml TSB tubes and four sterile 18 ml thioglycolate medium tubes. The same procedure is carried out for the sterile saline. Then, four tubes of TSB and thioglycolate medium containing test solution and saline each are placed in an incubator at 35-37°C and at room temperature. Blank tubes of each medium are included.
 - b. Four blood agar plates are streaked with 0.1 ml of test solution. Two plates are sealed in an anaerobic bag. Sterile saline (0.1 ml) is streaked on to 2 blood agar plates. Two blood agar plates are streaked with saliva to serve as positive controls. All plates are incubated at 35-37°C.
4. Four sterile tubes of each medium are inoculated with saturated cultures of B. subtilis, C. albicans and B. vulgatus. Four of each tubes are placed in an incubator and at room temperature. Two blood agar plates are streaked with saturated cultures of B. subtilis and C. albicans and four plates are streaked with B. vulgatus. Two plates are sealed in an anaerobic bag. All plates are placed in the incubator.
5. All cultures are checked after 24 hours of incubation and results reported to the Principal Investigator or his designee. The positive controls are discarded at this time. The rest of the blood agar plates and culture tubes are checked at 2 days, 5 days, 7 days and 14 days post culture initiation. Used and dried blood agar plates, if any, are disposed of as Regulated Medical Waste (see Dept. Guideline IC-6.2). The Principal Investigator, the Responsible Physician, or their designee should be notified of the results.

Records:

A flow sheet (Attachment 2, or equivalent), shall be used for each test batch. These documents shall provide a record of procedures, manufacturers, lot numbers and expiration dates, as well as, certification of sterility. The flow sheet may be filed in the patient's medical chart.

2.2 Pyrogen Testing

Testing shall be as required by FDA regulations. The following is the procedure to be followed for testing done on-site. Testing by independent vendors may be an acceptable alternative.

Equipment:

1. Laminar flow hood (certified per JCAHO Rules and Regulations).
2. Refrigerator (temperature monitored and recorded using NIST thermometer by authorized designee)
3. Freezer (temperature monitored and recorded using NIST thermometer by authorized designee)
4. Waterbath (temperature monitored and recorded using NIST thermometer by authorized designee)
5. Vortex Mixer

Materials:

1. Pyrogen standard: E. coli Endotoxin O55:B5, 10 ng., Lyophilized, BioWhittaker, Walkersville, MD.
2. Lysate: Limulus Amebocyte Lysate (LAL) lyophilized: BioWhittaker, Walkersville, MD
3. LAL Reagent Water: BioWhittaker, Walkersville, MD
4. Pyrogen free pipettes: Costar, Cambridge, MA
5. Microliter pipette (100l) + sterile tips: MLA, Mount Vernon, NY
6. Endotoxin Challenge Vials (ECV): BioWhittaker, Walkersville, MD
7. Pyrogen free test tubes: 10x75 mm and 13x100 mm, BioWhittaker, Walkersville, MD

NOTE: Alternative suppliers may be considered. All materials used must be within the expiration date.

Procedures:

NOTE: Persons designated to perform the Limulus Amebocyte Lysate (LAL) test must have appropriate knowledge of sterile techniques and bacteriology.

1. All procedures are to be carried out inside a certified laminar flow hood following the requirements of Dept. Guideline QAU-2.9.0, "Laminar Flow Hoods". All materials coming in contact with the test solution and test reagents must be certified pyrogen-free by the Endotoxin Challenge Vials.
2. The Control Std. Endotoxin (CSE) is reconstituted with 5.0 ml of LAL reagent H₂O and vortexed for at least 15 minutes. The LAL is reconstituted with 1.8 ml of LAL reagent H₂O and swirled gently for 30 seconds. Five vials of LAL are used for each pyrogenicity test. The two ECV vials are reconstituted with 1 ml of LAL reagent H₂O and vortexed for 30 minutes. (The ECV vials are delivered to the Bacteriology Laboratory together with the test solution).
3. Serial dilutions (covering the labeled sensitivity of the lysate) of the standard endotoxin (CSE) are made with LAL reagent water. Each dilution is vortexed for one minute and then transferred into quadruplicate 10x75 mm pyrogen free tubes. A negative control is set up using the LAL reagent water. The two ECV vials as well as an undiluted test solution sample are tested. The reconstituted lysate (100 l) is added 1:1 to all the tubes, swirled gently and placed in a 37°C water bath. The time of placement is recorded. This procedure is followed for each set of samples. Any vibration of the water bath should be avoided during the incubation period. In one hour ± two minutes of incubation each tube is checked for gelation. A firm gel, that holds its shape upon inversion is considered positive. No gel or a very light runny gel is considered negative.
4. a) A maximum valid dilution of test solution is prepared according to FDA guidelines (December 1987). This dilution is mixed with a serial dilution of the standard endotoxin and vortexed for 1 minute each. The serial dilution is transferred into small pyrogen free tubes and mixed 1:1 with the lysate. The tubes in quadruplicate are incubated in a water bath for 60 minutes ± 2 and each tube checked for gelation.
b) The results of the endotoxin in reagent water and of the test solution dilution should be within plus/minus a two-fold dilution of the labeled sensitivity. The endotoxin and lysate should be matched and a certificate of analysis available from the manufacturers. The Principal Investigator, the Responsible Physician or their designee, should be notified of the results.

Records:

A flow sheet (Attachment 3, or equivalent), shall be used for each test batch. These documents shall provide a record of procedures, manufacturers, lot numbers and expiration dates, as well as, certification of sterility. The flow sheet may be filed in the subject's medical chart.

The only official copy of this file is the one online at the Medical Department website under "Clinical Research Center Policy Manual." Before using a printed copy, verify that it is the most current version by checking the document effective date on the website.

Sterility and Pyrogenicity Testing may be conducted at the following firms:

Toxikon
15 Wiggins Avenue
Bedford, Ma., 01730
Tel.: (781)275-3330
1-800-458-4141

Leberco Laboratories
123 Hawthorne Street
Roselle Park, N. J. 07204
Tel.: (201)245-1933

Luizzi Microbiology Laboratory
272 Islip Avenue
Islip, N. Y. 117512
Tel.: (516)581-7379

Viromed Laboratories
2540 Executive Drive
St. Paul, MN. 55120

Any other accredited laboratory may be used.

STERILITY TEST FLOW SHEET

ID#

MANUFACTURER LOT NO. EXP. DATE

1. Place 0.1 ml injectate into 4 sterile broth tubes of 18 ml thioglycolate medium and 0.1 ml injectate into 4 sterile tubes of tryptic soy broth. Place 0.1 ml sterile saline into 4 thioglycolate and 4 tryptic soy broth tubes (negative control). Place 2 of each broth tubes in an incubator @ 35-37°C and 2 of each broth tubes @ room temp and 2 blank tubes @ 35-37°C and 2 @ room temp.

2. Spread 0.1 ml of injectate on 4 blood agar plates. Seal plates in anaerobic bags. Spread 0.1 ml sterile saline on 2 blood agar plates (negative control). Spread saliva on 2 blood agar plates (positive control). Place all plates sealed and unsealed in the incubator overnight @ 35-37°C plus 2 blank plates.

3. Inoculate 4 sterile tubes of each medium thioglycolate and tryptic soy broth with 0.1 ml of saturated cultures of Bacillus subtilis, Candida albicans, and Bacteroides vulgatus and place 2 tubes of each medium into the incubator and two at room temp (positive control).

4. Streak 2 blood agar plates with B subtilis and C. albicans and 4 blood agar plates with B. vulgatus. Seal 2 plates of B. vulgatus in anaerobic bags. Place all plates in incubator @ 35-37°C. (Positive controls).

5. Check all blood agar plates for growth @ 24 hours, ~2 days, 5 days, 7 days.

6. Check all broth tubes for growth @ 24 hrs, ~2 days, 5 days, 7 days and 14 days.

Certified sterile by: _____

Date:

PYROGEN TEST FLOW SHEET

ID#

MANUFACTURER LOT NO. EXP. DATE

1. Reconstitute pyrogen standard with 5.0 mL sterile H₂O and vortex vigorously for 15 mins.

2. Prepare a IEU/mL dilution of reconstituted endotoxin in sterile H₂O.

3. In 13 X 100 mm pyrogen-free sterile tubes, prepare a five serial 1:1 dilution of IEU/mL pyrogen std with sterile H₂O. Vortex 1 min after each dilution.

4. Dispense 4 X 100µL aliquots of each dilution into small 10 X 75 mm pyrogen free tubes. Add 4 tubes of sterile water + 4 tubes of test sample of appropriate dilution + 2 Endotoxin challenge vials (baked and unbaked) reconstituted with 1 mL sterile H₂O and vortexed for 30 min.

5. Reconstitute lyophilized Limulus Amebocyte Lysate by adding 1.8 ml of sterile H₂O to each vial. Swirl gently for ~30 seconds. Add 100 µl aliquots of LAL to the 100 µL aliquots of endotoxin-sterile H₂O dilutions plus H₂O and undiluted test solution. Vortex each set of tubes at once and place in water bath which is at 37°C and record time of placement.

6. Remove tubes from the H₂O bath at exactly 1 hr ± 2 min. of incubation. Check for gelation.

7. Prepare a serial dilution of IEU/mL pyrogen standard with an appropriate dilution of test solution instead of H₂O in the 13 X 100 mm pyrogen free tubes and vortex 1 min between each dilution.

8. Dispense 4 X 100 µL aliquots of each endotoxin-injectate dilution into 10 x 75 mm pyrogen free tubes. Add 4 tubes of H₂O and 4 tubes of diluted test solution.

9. Add 100 µL of reconstituted LAL to the 100 µL aliquots of endotoxin-test solution dilutions. Add H₂O and diluted test solution. Vortex each set of 4 tubes at once and place in H₂O bath which is at 37°C and record time of placement.

10. Repeat Step #6.

Certified pyrogen free by: _____

Date: