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Validation of Sterility Testing

Validation of Suitability for Radiopharmacy Sterility Testing

Media testing

pH Testing

Reference ATCC strains Preparation

Suitability (Growth Promotion) Testing

Validation (Bacteriostasis/Fungistasis) Testing

Validation of Suitability for Bone Bank Sterility Testing

Media testing

pH Testing

Reference ATCC strains Preparation

Suitability (Growth Promotion) Testing

Validation (Bacteriostasis/Fungistasis) Testing

Validation of Suitability for Pharmacy and Cell Therapy Products Sterility Testing by BacT/Alert Dual T System

Media testing

Reference ATCC strains Preparation for each organism

Suitability (Growth Promotion) Testing

Validation (Bacteriostasis/Fungistasis) Testing

Record of Edited Revisions
Biological Samples

Bone Bank Specimens

I. Introduction

Bone specimens and swabs from Bone Bank are submitted for sterility check. Positive controls swabs are submitted routinely as a process control sample for swab handling. These specimens are cultured for 7 days before a final report is issued.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

Inoculate specimen into a Fastidious Anaerobic Broth.

<table>
<thead>
<tr>
<th>Culture:</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>O₂, 35°C x 7 days</td>
<td></td>
</tr>
</tbody>
</table>

IV. Isolation and Identification

Read cultures daily for 7 days (excluding weekends)

On turbid Fastidious Anaerobic Broths, prepare smear for Gram stain and sub-culture onto:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 48 hours (examine at 24 and 48 hours)</td>
</tr>
<tr>
<td>Brucella Agar (BRUC)</td>
<td>ANO₂, 35°C x 48 hours (examine at 48 hours)</td>
</tr>
</tbody>
</table>

For specimens: Identify all isolates.
For controls: Visual growth of oral flora. No work up required.
V. **Sensitivity Testing**

Not required.

VI. **Reporting**

Telephone all positive results.

Interim Report:
- **Negative Report:** "No Growth"
- **Positive Report:** Report all isolates without quantitation.
- **Control Report:** “Oral flora”

VII. **Reference**

American Association of Tissue Banking Standards
Bone Bank Specimens - Fresh Osteochondral Allograft

I. Introduction

Fresh allograft bone specimens and swabs from Bone Bank are submitted for sterility check. These specimens are cultured for 7 days. However, these fresh allografts may be transplanted before the final report is issued.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

Inoculate specimen into a Fastidious Anaerobic Broth. Place a red dot onto the cap of the broth.

Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>O₂, 35°C x 7 days</td>
</tr>
</tbody>
</table>

IV. Isolation and Identification

Read cultures twice daily at 8:00 am and 3:00 p.m. for 7 days. Additional readings will be required when a recipient is located AND 15 to 20 minutes prior to transplant in the OR. Document all readings in the LIS.

On turbid Fastidious Anaerobic Broths, prepare smear for Gram stain and sub-culture onto:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 48 hours (examine at 24 and 48 hours)</td>
</tr>
<tr>
<td>Brucella Agar (BRUC)</td>
<td>ANO₂, 35°C x 48 hours (examine at 48 hours)</td>
</tr>
</tbody>
</table>
Identify all isolates.

V. **Sensitivity Testing**

Not required.

VI. **Reporting**

Preliminary Report:
- Negative Report: “No growth to date, further report to follow” Status as preliminary (^P) after every reading.
  Telephone all positive reports to the Bone Bank.

Interim Report:
- Negative Report: "No Growth"
  Telephone all positive reports to the Bone Bank.

VII. **Reference**

American Association of Tissue Banking Standards
Cardiovascular Lab Specimens (Dog)

Introduction

These specimens are collected from the research laboratory. Dr. Wilson is the contact person (ext. 4795).

Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

Processing of Specimens

i) Direct examination: Gram stain

ii) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>AnO₂, 35°C x 7 days</td>
</tr>
</tbody>
</table>

Isolation and Identification

All isolates are to be identified. Prepare Gram stain smear and subculture all turbid THIO.

Sensitivity Testing

Not required.

Reporting

Telephone all positive reports to ward / physician.

Interim Report:

Negative Report: "No Growth"

Positive Report: Report all isolates
Medicinal Leech Testing

Introduction

Medicinal leeches permit enhance venous outflow post plastic and reconstructive surgery to salvage tissue flaps, grafts or replants when tissue viability is threatened by venous congestion. Their use is associated with an increased risk of infection due to *Aeromonas hydrophila*, *Aeromonas veronii* or other less commonly isolated aerobic organisms (*Serratia* spp, *Proteus* spp, *Morganella* spp, *Vibrio* spp, *Pseudomonas* spp) found in the leech’s normal flora. In cases of suspected infection, the leech will be submitted for culture and susceptibilities.

Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

Processing of Specimens

Leech specimen:
Remove any large amounts of leech fluid from within the sterile container containing the leech. Transfer broth contents of two Thioglycollate broths into the sterile container.

Leech storage fluid:
Aseptically transfer 1.0mL of fluid into Thioglycollate broth.

Leech vendor fluid:
Aseptically transfer 1.0mL of fluid into Thioglycollate broth.

Leech storage tank swab:
Eswab: Aseptically transfer contents of eswab fluid to a Thioglycollate broth.

Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioglycolate Broth (THIO)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)*</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Colistin Nalidixic Acid Agar (CNA)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>
Isolation and Identification
Examine Thioglycollate daily for two days.

Subculture all turbid broths onto MacConkey Agar, Colistin Nalidixic Acid Agar, and Chocolate Agar. *Apply a ciprofloxacin and Trimethoprim/Sulfamethoxazole disk to the main inoculum of the MacConkey Agar.

Incubate media as above examining MAC, CNA, CHOC after 24 and 48 hours incubation.

For colonies growing on MacConkey Agar, work on colonies closest to the Ciprofloxacin Trimethoprim/Sulfamethoxazole disks for susceptibility testing to aid in identification of any multidrug resistant Aeromonas spp. within the culture.

All aerobic isolates are to be identified and frozen.

Sensitivity Testing
Refer to Susceptibility Testing Manual

Reporting
Preliminary Report:

Negative Report: “No growth to date, further report to follow”

Interim Report:

Negative Report: "No Growth"
Report with appropriate susceptibilities.
References


Tissue Cultures Specimens for Injection

I. Introduction

Samples of in vitro cell cultures are submitted for sterility check prior to injection into humans.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

iii) Direct examination: Gram stain (if requested)

iv) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>MacConkey Agar (MAC)</td>
<td>O₂, 35°C x 48 hours</td>
</tr>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>AnO₂, 35°C x 14 days</td>
</tr>
</tbody>
</table>

IV. Isolation and Identification

All isolates are to be identified. Prepare Gram stain smear and subculture all turbid THIO.

V. Sensitivity Testing

Not required.

VI. Reporting

Telephone all positive reports to ward / physician.

Preliminary Report:

Negative Report: “No growth to date, further report to follow”

Interim Report: “No growth after 14 days.”
Positive Report: Report all isolates
Non-biological Specimens

Air Sampling by Air Flow Sampling Apparatus

I. Introduction

Air sampling specimens are collected for the purpose of compliance to Clean Air Standard or in case of patient care areas, the Air-Borne Fungal Spore Level. Various apparatus can be used for sampling. The amount of air required to sample will depend on the standard set for the purpose of the particular area. The media used will also depend on the purpose of the area to be measured and the type of organisms to be counted. Culture media that has been subjected to a specified volume of airflow will be submitted to the microbiology lab for incubation and colony count.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

1. Incubate culture media received at 37°C for 48 hours if bacteria count is required. Incubate culture media at 30°C for 7 days if fungal culture is required. Examples of culture media used:

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Blood Agar</td>
<td>37°C x 48 hours</td>
</tr>
<tr>
<td>Fungi</td>
<td>Inhibitory Mold Agar</td>
<td>30°C x 7 days</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Trypticase Casein Agar</td>
<td>37°C x 48 hours</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td>30°C x 7 days</td>
</tr>
<tr>
<td>Fungi</td>
<td>Rose Bengal Agar</td>
<td>30°C x 7 days</td>
</tr>
</tbody>
</table>

IV. Isolation and Identification

1. At the end of the required incubation period, perform a total colony count per media.
2. If air flow rate and sampling time was given, calculate the colony forming units per cubic meter of air sampled as follows:
   
   Flow rate = a L/min.
Sampler running time = \( b \) minutes
Volume of air sampled = \( a \times b \) L = \( \frac{ab}{1000} \) m\(^3\) = \( d \) m\(^3\)
Bacterial or mould count = \( c \) CFU
Total CFU/m\(^3\) air sampled = \( \frac{c}{d} \) CFU/m\(^3\) air

3. Identify organism only if requested.

V. **Sensitivity Testing**

Not required.

VI. **Reporting**

Interim Report:

If airflow rate information is not provided, report as:
“Bacterial colony count at incubation temperature is \( X \) CFU”
“Mould colony count at incubation temperature is \( X \) CFU”

If airflow information is provided, report as per calculated CFU/m\(^3\):
“Bacteria colony count \( X \) CFU/m\(^3\)”
“Mould colony count \( X \) CFU/m\(^3\)”

VII. **Reference**

I. **Introduction**

The Attest is a biological indicator used for optimum quality control of steam or gas sterilization. Ampoule (green top) for gas sterilization contains *Bacillus subtilis*. Ampoule (brown top) for steam sterilization contains *Bacillus stearothermophilus*.

II. **Specimen Collection and Transport**

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. **Procedure**

The Attest must be activated by crushing the media-containing inner glass ampoule.

1. With the ampoule tilted slightly toward you, place the bottom of the ampoule into the 3M Attest dry heating block.

2. Push the ampoule straight back into an upright position. This activates the indicator.

3. Push the crushed ampoule down to firmly seat it in the 3M heating block.

4. Incubate for 48 hours and read each ampoule as follows:

<table>
<thead>
<tr>
<th></th>
<th>STEAM ATTEST</th>
<th>FLASH ATTEST</th>
<th>GAS ATTEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap Colour</td>
<td>Brown</td>
<td>Blue</td>
<td>Green</td>
</tr>
<tr>
<td>Incubation Temp.</td>
<td>56°C</td>
<td>56°C</td>
<td>37°C</td>
</tr>
<tr>
<td>Negative Colour</td>
<td>Purple</td>
<td>Purple</td>
<td>Green</td>
</tr>
<tr>
<td>Positive Colour</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

IV. **Reporting**

All positive results, excluding control, must be phoned to ward / department and to Infection Control.
Interim Reports:

Negative Report: "Test spores: No growth" or "Test spores: No growth
Control spores: GROWTH"

Positive Report: "Test spores: GROWTH" or "Test spores: GROWTH
Control spores: GROWTH / No growth"
Chemspore / Sterikon

I. Introduction

A chemical and biological indicator used for monitoring steam sterilization processes in wet environments (washer/sterilizer) when a "spore strip" type of sterility indicator cannot be used.

The Chemspore ampoule contains a thermal-sensitive chemical process indicator inside an inner glass tube. The chemical melts and changes colour when minimal heat is applied. The ampoule also contains spores of Bacillus stearothermophilus suspended in a bacteriological growth medium containing a pH indicator.

Sterikon ampoule consists of an ampoule that contains nutrient broth, pH indicator and spores of Bacillus stearothermophilus.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Procedure

1. Place ampoule in the Chemspore or Sterikon incubator preset at 56°C. An unexposed (control) ampoule should also be incubated along with the exposed ampoule as a control.

2. Examine ampoules after 24 and 48 hours. The control ampoule medium should turn bright yellow and turbid, indicating viable microorganisms after 24 hours. If it does not turn yellow after 24 hours, check incubator temperature (56°C-65°C). The test ampoule should be clear with no change in colour, indicating that sterilization has been achieved.

IV. Reporting

All positive test results must be phoned to the ward / department and to Infection Control.

Interim Reports:

Negative Report: "Test spores: No growth" or "Test spores: No growth
Control spores: GROWTH"
Positive Report: "Test spores: GROWTH" or
"Test spores: GROWTH
Control spores: GROWTH / No growth"
Contact Lens & Solution

I. Introduction

Contact lenses and solutions may be submitted to the Microbiology laboratory for detection of contamination including the presence of Acanthamoeba.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure

A. Processing of Specimens:

NB: If previously inoculated plates received and no specimen or swab received, then direct examination is not performed.

a) Direct Examination: Gram stain – Quantitate the presence of pus cells and organisms.

Calcofluor white stain. (If two smears are provided) - Refer to Mycology Manual.
b) Culture:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (BA)</td>
<td>CO₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>O₂, 35°C x 5 days</td>
</tr>
<tr>
<td>Inhibitory Mold Agar (IMA)*</td>
<td>O₂, 30°C x 3 weeks</td>
</tr>
</tbody>
</table>

*Forward the fungal culture media to the Mycology section for incubation and workup.

B. Interpretation of Cultures:

Examine the culture plates daily. If no growth on culture plates but growth in THIO, perform Gram stain and sub-culture THIO onto BA, and CHOC and incubate x 48 hours.

Work up all isolates other than skin flora.

C. Susceptibility Testing:

Refer to Susceptibility Testing Manual.

V. Reporting Results

Preliminary Report:

Negative Report: “No growth to date, further report to follow” Status as preliminary (^P) after every reading.

Positive report: All isolates with appropriate sensitivities without quantitation.

Interim Report:

Negative Report: "No Growth"

Positive report: All isolates with appropriate sensitivities without quantitation.
Distilled/De-Ionized Water Sterility

I. Introduction

Distilled or de-ionized water samples are submitted for colony count to check for suitability as reagent water in clinical laboratories.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCM02001

III. Processing of Specimens

1. Process sample within one hour of sampling or refrigerate up to 6 hours.
2. Vortex sample for 10 seconds.
3. Inoculate 1 mL of sample onto a BHI Agar plate and spread the inoculum over the entire agar surface.
4. Incubate the BHI plate at 35°C x 24 hrs
5. Remove the plate from the incubator and incubate the plate at room temperature for an additional 24 hours.
6. Count and record the number of colonies on the entire agar surface.

IV. Reporting

Interim Reports:

- Negative Report: “No Growth”
- Positive Report: Report the number of colonies recorded as “x CFU/mL”
Endoscope Surveillance Samples

I. Introduction

Endoscopes (colonoscopies, gastroscopes and duodenoscopes) are instruments that require high level disinfection and will be tested for microbial bioburden to assess cleaning and disinfection practices. Results will determine the need to repeat reprocessing, removal from use or repair.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

Vortex conical tube containing the endoscope flushed saline and a flocked swab (2 swabs if from duodenoscope) for 30 seconds at 10 second bursts.

Inoculate 1mL of specimen into 10mL BHI broth for overnight incubation on shaker in O₂ at 35°C.

After incubation, inoculate 1mL of the BHI broth onto one blood agar plate. Spread evenly over plate.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHI Broth</td>
<td>O₂, 35°C Overnight</td>
</tr>
<tr>
<td></td>
<td>Blood Agar (BA)</td>
<td>O₂, 35°C x 24 hours</td>
</tr>
</tbody>
</table>

IV. Isolation and Identification

Read blood agar plate after 24 hours for any growth.
All isolates require minimal identification e.g. Gram negative bacilli, *Enterococcus* species, *CNST*, *Bacillus* species, *Corynebacterium* species, Gram positive bacilli, mould, etc.

V. **Sensitivity Testing**

Not required.

VI. **Reporting**

Interim reports:

- **Negative Report:** Final Report: “No growth”
- **Positive Report:** Report all isolates without quantitation.
Environmental Monitoring

Introduction

Environmental samplings are collected for the purpose of detecting contamination of a clean area caused by aerosol or procedural techniques. The media used will depend on the area to be assessed and the type of organisms to be counted. Culture media plates are exposed to air, surfaces such as equipment and/or glove prints of staff while media fill broths are manipulated to simulate compounding conditions. The exposed culture media are submitted to microbiology for incubation and colony count.

Specimen Collection

Air Sampling
Surface Sampling
Gloved Fingertip Sampling
Media Fill

See Pre-analytical Procedure – Specimen Collection QPCM102001

Culture Processing

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air Sampling</td>
<td>Bacteria</td>
<td>Tryptone Soya Agar</td>
</tr>
<tr>
<td>Surface Sampling</td>
<td>Bacteria</td>
<td>Tryptone Soya Agar (with lecithin and polysorbate) - 55mm plate</td>
</tr>
<tr>
<td>Gloved Fingertip Sampling</td>
<td>Bacteria</td>
<td>Tryptone Soya Agar (with lecithin and polysorbate)</td>
</tr>
</tbody>
</table>

For High Risk Compounding add:

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td>Inhibitory Mold Agar</td>
<td>O₂ 30°C x 7days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media Fill</td>
<td>Bacteria</td>
<td>Vial with Tryptone Soya Broth</td>
</tr>
</tbody>
</table>
Interpretations

A. Sampling plates:

<table>
<thead>
<tr>
<th>GMP Grade</th>
<th>Settle plates (90mm) cfu/4hrs</th>
<th>Contact plates (55mm) cfu/plate</th>
<th>Glove prints (5 fingers) cfu/glove</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>50</td>
<td>-</td>
</tr>
</tbody>
</table>

1. At the end of each temperature incubation period, perform a total colony count per media.
2. Identify any amount of organism to genus level.
3. Clean area if colony count is >1. Biosafety cabinets are GMP grade A.

B. Media Fill

1. Observe the Tryptone Soya Broth daily (Monday to Friday) for turbidity, record in LIS.

Reporting

A. Sampling plates:

Report only TOTAL colony count for each incubation temperature, listing all organisms at minimum to the genus level.

i) Preliminary Report:

Plates with No Growth

~ No growth to date, further report to follow.

Plates with growth:

“Total bacterial colony count at 35°C is ## CFU including list all organism isolated.”

Update result daily as necessary.
ii) Interim Report:

Plates with growth:

“Total bacterial colony count at 35°C is ## CFU including list all organism isolated.”
“Total mould colony count at 30°C is ## CFU including list all organism isolated.”

Plates with No Growth:

“Total bacterial colony count at 35°C is 0 CFU”
“Total mould colony count at 30°C is 0 CFU”

B. Media Fill:

Interim Report:

Turbidity seen: “Passed - Sterile at 35C incubation temperature after 14 days incubation”

Failed “Failed - Turbid at 35C incubation temperature”

References


http://www.hc-sc.gc.ca/dhp-mps/compli-conform/gmp-bpf/docs/qui-0001-eng.php#sterile

2017. USP Compounding Compendium. The United States Pharmacopeial Convention, Rockville, MD

Hemodialysis Water Sterility

I. Introduction

Water samples from hemodialysis machines are submitted for colony count to check for sterility.

II. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. Processing of Specimens

1. Note the collection time of the sample.
2. Process the sample within 30 minutes of collection or refrigerate for up to 24 hours of collection.
3. Vortex sample for 10 seconds.
4. Inoculate 1 mL of sample onto a R2A plate.
5. Plate and spread the inoculum over the entire agar surface.
6. Incubate the R2A plate at room temperature (17-23°C) x 7 days
7. Count and record the number of colonies on the entire agar surface each day.
   Send a prelim report every day of testing. Send a final report on day 7.

IV. Reporting

Preliminary Report: “~No growth to date, further report to follow”
   “__ x CFU/mL”

Interim Reports:

   Negative Report: "No Growth"
   Positive Report: Report the number of colonies recorded as “x CFU/mL”

V. References

CAN/CSA-ISO 11663:15, Quality of dialysis fluid for haemodialysis and related therapies, [ISO 11663:2014, IDT], National Standard of Canada
I. **Introduction**  
Specimens such as soap, gel, India ink, talcum powder referred-in from other departments for sterility testing are cultured for 7 days before a final report is issued.

II. **Specimen Collection and Transport**  
See Pre-analytical Procedure – Specimen Collection QPCMI02001

III. **Processing of Specimens**  
Inoculate up to 1 mL of specimen into a Fastidious Anaerobic Broth. Read cultures daily for 7 days. Read cultures daily for 14 days if specimen is from the P.E.T. Centre at CAMH.  
**Culture:**

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fastidious Anaerobic Broth (THIO)</td>
<td>O₂, 35°C x 7 days</td>
</tr>
<tr>
<td></td>
<td>O₂, 35°C x 14 days (PET centre only)</td>
</tr>
</tbody>
</table>

IV. **Isolation and Identification**  
Prepare Gram stain smear and subculture all turbid Fastidious Anaerobic Broths. All isolates require minimal identification e.g. *Enterococcus* species, *Enterobacter* species, Gram negative bacilli, *Corynebacterium* species, Gram positive bacilli, mould, etc.

V. **Sensitivity Testing**  
Not required.

VI. **Reporting**  
Telephone positive reports if requested

Preliminary Reports:

Negative Report: “No growth to date, further report to follow” Status as preliminary (^P) after every reading

Interim Report:

Negative Report: “No growth after 14 days.”
Product Sterility Samples

I. **Introduction**

Sterility testing of products including pharmacy, cell therapy and other sterile products are performed to ensure safety of all products prior to use in patients. Specimen collection and inoculation into testing broths (Thioglycollate and Tryptone Soya) are done by the pharmacy and sent to Microbiology for incubation and culture processing. The microbiology laboratory is not permitted to inoculate pharmacy products into the testing broths in accordance to the exemption as outlined in the Health Canada issuance permit for sterility testing.

II. **Materials**

Non-radioactive pharmaceutical product
Thioglycollate broth (Oxoid MT2030) (10mL) tube *
Tryptone Soya broth (Oxoid MT2065) (10mL) tube *
Syringes (3mL)
Alcohol wipes

*Microbiology will send Thioglycollate and Tryptone Soya broths to pharmacies with a Certificate of Analysis from Oxoid
*Store broths at 2 - 8°C

III. **Specimen Collection and Transport (by facility)**

See Pre-analytical Procedure – Specimen Collection QPCMI02001

IV. **Processing of Specimens (by Microbiology section IV to VIII)**

1. On receipt, using the EPR specimen number, accession in LIS inoculated Thioglycollate (TH14) and Tryptone Soya (SD14) broths.

2. Label the Thioglycollate broth and the Tryptone Soya broth tubes with the corresponding LIS number

3. Inspect samples for rejection criteria as defined in Specimen Rejection Criteria manual MI_SM_RJCT.

**Note:** Notify and return (if required by client) all products not received in Thioglycollate or Tryptone Soya broths or rejected for other reason.
Only products inoculated in Thioglycollate and Tryptone Soya broths are to be accepted for sterility testing.

4. Incubate the broths as below:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioglycollate Broth (TH14)</td>
<td>O₂, 35°C x 14 days</td>
</tr>
<tr>
<td>Tryptone Soya Broth (SD14)</td>
<td>O₂, RT°C x 14 days</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 2 days</td>
</tr>
<tr>
<td>Fastidious Anaerobic Agar (BRUC)</td>
<td>AnO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

V. **Isolation and Identification**

Exam Thioglycollate broth and Tryptone Soya broth daily (Monday to Friday), record in LIS

Prepare Gram stain smear and subculture all turbid broths onto Chocolate (CHOC) and Fastidious Anaerobic (BRUC) media within the designated Class II BSC. Incubate media as above and exam CHOC daily for 2 days and BRUC after 48hrs incubation

All isolates are to be identified.

*For broths received turbid*

Process as above if evidence of increased turbidity is noted.

For material rendering the broths turbid creating inability to determine presence or absence of growth by visual examination at 14 days:

1. Transfer a portion (no less than 1mL) of each broth into a fresh tube of each respective broth
2. Continue to incubate original broths and transfer broth for 4 additional days.
3. If no evidence of microbial growth is found, the product complies and is negative.

For all positive samples, follow [Investigation of positive cultures](#) below and report appropriately.

VI. **Sensitivity Testing**
VII. Reporting

Telephone positive report(s) to submitting facility

Preliminary Reports:
Negative Report: “No growth to date, further report to follow” Status as preliminary (^P) after every reading

Final Report:
VIII. **Investigation of positive cultures**

1. Telephone positive(s) to respective pharmacy and send preliminary report(s) in LIS

2. Inform QA technologist of positive results. QA technologist to complete **INVESTIGATION OF OUT OF SPECIFICATION RESULTS** form and email to dispensing facility designated person.

3. A repeat sample will be sent by dispensing facility.

4. Pharmacy will complete and file the **INVESTIGATION OF OUT OF SPECIFICATION RESULTS** and follow their own protocol in the investigation of positive results.
### INVESTIGATION OF OUT OF SPECIFICATION RESULTS

**PRODUCT:**

To be completed by the Department of Microbiology and emailed to the Dispensing Facility:

<table>
<thead>
<tr>
<th>Testing Facility</th>
<th>Result</th>
<th>Media Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Number</td>
<td></td>
<td>Thioglycolate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tryptone Soya Broth</td>
</tr>
<tr>
<td>Original Sample</td>
<td></td>
<td>Lot Number:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Expiration Date:</td>
</tr>
<tr>
<td>QC temperature data within range:</td>
<td>Yes ☐ No ☐</td>
<td></td>
</tr>
<tr>
<td>Sterility testing performed as per protocol:</td>
<td>Yes ☐ No ☐</td>
<td></td>
</tr>
<tr>
<td>QA Technologist Signature:</td>
<td>Date:</td>
<td></td>
</tr>
</tbody>
</table>

To be completed and kept on record by the Dispensing Facility:

<table>
<thead>
<tr>
<th>Repeat Sample</th>
<th>Lot Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated Sample</td>
<td>Expiration Date:</td>
</tr>
<tr>
<td>Product Compliant:</td>
<td>Release product</td>
</tr>
<tr>
<td>No ☐ Action:</td>
<td></td>
</tr>
</tbody>
</table>

Pharmacy Technician / Pharmacist Signature: Date:
IX. Reference


2016. USP Compounding Compendium. The United States Pharmacopeial Convention, Rockville, MD

Pharmacy and Cell Therapy Products Sterility Testing by BacT/Alert Dual T System

I. Introduction

Sterility testing of pharmacy and cell therapy products is performed to ensure safety of all products prior to use in patients. Specimen collection and inoculation into testing broths (BacT/Alert iFA Plus and iFN Plus bottles) are done by the submitting facility and sent to the microbiology laboratory for incubation and culture processing. The microbiology laboratory is not permitted to inoculate sterile products into the testing broths in accordance to the exemption as outlined in the Health Canada issuance permit for sterility testing.

II. Materials

BacT/Alert iFA Plus and iFN Plus bottles

*Microbiology will send BacT/Alert iFA Plus and iFN Plus bottles to facilities with a Certificate of Analysis from Biomérieux.
*Store broths at room temperature

III. Specimen Collection and Transport (by facility)

See Pre-analytical Procedure – Specimen Collection QPCMI02001

IV. Processing of Specimens (by Microbiology section IV to VIII)

1. On receipt, accession the samples into the LIS and label the paired iFA Plus and iFN Plus with corresponding LIS numbers

2. Inspect samples for rejection criteria as defined in Specimen Rejection Criteria manual MI_SM_RJCT.

3. Load the bottles into the BacT/Alert 3D as follows:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFA Plus (Aerobic Bottle)</td>
<td>BacT/Alert 3D</td>
</tr>
<tr>
<td>iFN Plus (Anaerobic Bottle)</td>
<td>BacT/Alert 3D</td>
</tr>
<tr>
<td></td>
<td>20-25°C x 7 days</td>
</tr>
<tr>
<td></td>
<td>30-35°C x 7 days</td>
</tr>
</tbody>
</table>
Isolation and Identification

Process all bottles flagged positive by the BacT/Alert as follows:

Prepare Gram stain smear and subculture onto Blood Agar (SUBBA), MacConkey (SUBMC), Chocolate Agar (SUBCH) and Fastidious Anaerobic (SUBBR) media within the designated Class II BSC and incubate follows:

<table>
<thead>
<tr>
<th>Media</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Agar (SUBBA)</td>
<td>CO₂, 35°C x 2 days</td>
</tr>
<tr>
<td>MacConkey (SUBMC)</td>
<td>O₂, 35°C x 2 days</td>
</tr>
<tr>
<td>Chocolate Agar (CHOC)</td>
<td>CO₂, 35°C x 2 days</td>
</tr>
<tr>
<td>Fastidious Anaerobic Agar (BRUC)</td>
<td>AnO₂, 35°C x 48 hours</td>
</tr>
</tbody>
</table>

Exam SUBBA, SUBMC, SUBCH daily for 2 days and SUBBR after 48hrs incubation

All isolates are to be identified.

For all positive samples, follow [Investigation of positive cultures](#) below and report appropriately.

V. Sensitivity Testing

Not required

VI. Reporting

Telephone positive report(s) to submitting pharmacy

Preliminary Reports:

Negative Report: “No growth to date, further report to follow” Status as preliminary (^P) after every reading

Interim Report:

Negative Report: “No growth after 7 days.”

VII. Investigation of positive cultures

1. Telephone positive(s) to respective pharmacy and send preliminary report(s) in LIS

2. Inform QA technologist of positive results. QA technologist to complete INVESTIGATION OF OUT OF SPECIFICATION RESULTS form and email to dispensing facility designated person.

3. A repeat sample will be sent by dispensing facility.

4. Facility will complete and file the INVESTIGATION OF OUT OF SPECIFICATION RESULTS and follow their internal protocol to complete the investigation of positive results.
### INVESTIGATION OF OUT OF SPECIFICATION RESULTS

**PRODUCT:**

To be completed by the Department of Microbiology and emailed to the Dispensing Facility:

<table>
<thead>
<tr>
<th>Original Sample</th>
<th>Testing Facility Reference Number</th>
<th>Result</th>
<th>Media Information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| QC temperature data within range: | Yes ☐ No ☐ |
| Sterility testing performed as per protocol: | Yes ☐ No ☐ |
| QA Technologist Signature: | Date: |

To be completed and kept on record by the Dispensing Facility:

<table>
<thead>
<tr>
<th>Repeat Sample</th>
<th>Lot Number:</th>
<th>Explication Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Uninoculated Sample</th>
<th>Lot Number:</th>
<th>Explication Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Product Compliant: | Yes ☐ Release product | No ☐ Action: |

Pharmacy Technician / Pharmacist Signature: Date:

---

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X. Reference


2016. USP Compounding Compendium. The United States Pharmacopeial Convention, Rockville, MD

**Spore Strip**

**I. Introduction**

A spore strip is used for monitoring steam sterilization (autoclave), chemical vapour sterilization (chemiclave) or radiation processes. The spore strip is embedded with spores of *Bacillus stearothermophilus* (for autoclave), *Bacillus subtilis* (for chemiclave) or *Bacillus pumilus* (for radiation). The spore strip is put into the sterilizer along with the load of materials to be sterilized.

**II. Specimen Collection and Transport**

See Pre-analytical Procedure – Specimen Collection QPCMI02001

**III. Procedure**

1. With aseptic technique, transfer spore strip to a 1-mL Trypticase Soy Broth tube.
2. If a control strip is received, transfer the control strip to another 1-mL Trypticase Soy Broth tube.
3. Incubate the Trypticase Soy Broth as follows:
   - Check the sterilization method written on the specimen label or the requisition.

<table>
<thead>
<tr>
<th>Sterilization Method</th>
<th>Incubation Temperature</th>
<th>Length of Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclave</td>
<td>56°C heating block</td>
<td>7 days</td>
</tr>
<tr>
<td>Statim autoclave</td>
<td>56°C heating block</td>
<td>7 days</td>
</tr>
<tr>
<td>Midmark Ultraclave</td>
<td>56°C heating block</td>
<td>7 days</td>
</tr>
<tr>
<td>Chemiclave</td>
<td>35°C incubator</td>
<td>7 days</td>
</tr>
<tr>
<td>Radiation (primarily from Bone Bank)</td>
<td>35°C incubator</td>
<td>7 days</td>
</tr>
</tbody>
</table>

4. Examine the TSB daily for 7 days.
5. Confirm growth of Bacillus by performing a gram smear on turbid broths.

**Note:** Send broth to the Provincial Health Lab for identification if requested.

**IV. Reporting**

All positive test results must be phoned to the ward / department.

Interim Reports:

Negative Report:  "Test spores: No growth" or "Test spores: No growth
Control spores: GROWTH"

UNIVERSITY HEALTH NETWORK/MOUNT SINAI HOSPITAL, DEPARTMENT OF MICROBIOLOGY

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NOTE: This document is Uncontrolled When Printed.
Any documents appearing in paper form that do not state “CONTROLLED COPY” in red print are not controlled and should be checked against the document (titled as above) on the server prior to use.
Positive Report:        "Test spores: GROWTH" or
                        "Test spores: GROWTH
Control spores: GROWTH / No growth"
Validation of Sterility Testing

Validation of Suitability for Product Sterility Testing

I. Introduction

A sterility test is technically not valid unless a Suitability Test and a Growth Promotion Test (Bacteriostasis and Fungistasis Test) are performed as per (USP) United States Pharmacopeia <71> guidelines. The suitability test determines that the test sample does not possess any inhibiting factors to the growth of less than 100 viable microorganisms in the test media and cause a false negative sterility test. The growth promotion test confirms that each lot of growth media used will support the growth of less than 100 viable microorganisms.

II. Reagents/Materials/Media

Media

In accordance with USP <71> guidelines, commercially prepared Soy-bean casein digest (SCD) media and fluid Thioglycollate media (FTM) can be use for sterility testing. (If testing media are prepared in-house, samples must be selected from every load sterilized for testing and pH check).

Reference ATCC strains

Table 1: Reference strains for Suitability (Growth Promotion) & Validation

<table>
<thead>
<tr>
<th>Type</th>
<th>Organism</th>
<th>Reference strain</th>
<th>Incubation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td>Staphylococcus aureus</td>
<td>ATCC 6538</td>
<td>30-35°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 9027</td>
<td>30-35°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td>Bacillus subtilis</td>
<td>ATCC 6633</td>
<td>20-25°C for 24 hours</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>Clostridium sporogenes</td>
<td>ATCC 19404</td>
<td>30-35°C for 48 hours</td>
</tr>
<tr>
<td>Fungi</td>
<td>Candida albicans</td>
<td>ATCC 10231</td>
<td>20-25°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td>Aspergillus brasiliensis/niger</td>
<td>ATCC 16404</td>
<td>20-25°C for 3 days</td>
</tr>
</tbody>
</table>

Sampling of lots

Samples for sterility testing are submitted by the facility with minimum quantity of product to be tested from each container as per USP <71> table 2.

Table 2: Minimum quantity to be tested from each container
<table>
<thead>
<tr>
<th>Product type</th>
<th>Product Quantity</th>
<th>Minimum inoculum for each medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids</td>
<td>&lt;1mL</td>
<td>Whole content</td>
</tr>
<tr>
<td></td>
<td>1- 40 mL</td>
<td>Half the contents but not &lt;1mL</td>
</tr>
<tr>
<td></td>
<td>41 – 100 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td></td>
<td>&gt;100 mL</td>
<td>10% contents but not &lt;20mL</td>
</tr>
</tbody>
</table>

**Note:** Volume of sample under test must be ≤10% of media i.e. 90% medium and 10% product

### III. Procedure

Test methods for Suitability and Growth Promotion on are done by the direct transfer of the product and/or reference organisms into the fluid thioglycollate medium (FTM) and the soybean casein digest medium (SCD).

**Media testing**

For in-house prepared media, each sterilized load of medium must be tested for pH, sterility and growth promotion. For commercially prepared media, a certificate of growth promotion must accompany the media if the media is to be exempted from repeat testing by sterility testing laboratory.

**pH Testing**

*Table 3: pH ranges for Thioglycollate fluid and Soybean – Casein Digest*

<table>
<thead>
<tr>
<th>Medium</th>
<th>pH after sterilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid Thioglycollate</td>
<td>7.1 ± 0.2</td>
</tr>
<tr>
<td>Soybean-Casein Digest</td>
<td>7.3 ± 0.2</td>
</tr>
</tbody>
</table>

**Reference ATCC strains Preparation**

Reconstitute microorganisms as per manufacturer’s insert. Subculture to non-selective agar and incubate as per *Table 1*. Subcultured isolates are stored in trisodium citrate glycerol at -70°C as stock cultures. Monthly, stock cultures are subcultured to non-selective agar and then to Trypticase Soy Agar slope (TSA) as working culture.

Prepare a working suspension of 100 CFU/mL of microorganism:

1. Subculture from the TSA slope to Blood agar plate and incubate as per *Table 1*
2. Prepare a standardized 0.5 McFarland (1 x 10^6 CFU/mL) of the 24 hours culture in 9.9 mL saline
3. Pipette 0.1mL of the 0.5 McFarland suspension into 9.9 mL saline to obtain 1:100 dilution (A) (1 x 10⁶ CFU/mL)
4. Pipette 0.1mL of (A) into 9.9 mL saline to obtain 1:10,000 dilution (B) (1 x 10⁴ CFU/mL)
5. Pipette 0.1mL of (B) into 0.9mL saline to obtain 1:100,000 dilution (C) (1 x 10³ CFU/mL)
6. Dispense 0.1mL of final dilution (C) to blood agar plate and perform colony count to confirm final concentration of 100 CFU

**Suitability (Validation) Testing**

Suitability testing is performed for all new products and re-validated when there’s a change in procedure or protocol. Inoculum of 10 – 100 CFU/mL of reference organism is added directly to the testing media which contains the testing product. Test is valid if the challenge organism show visible growth in the test media containing product, within 3 days for bacteria and within 5 days for fungi.

For each specimen, pipette 1ml of the specimen into each of the media. To each tube then add 100 uL of the 1 x 10⁴ CFU/mL of the respective reference microorganisms.

*Refer to Table 4*

**Growth Promotion (Bacteriostasis/Fungistasis) Testing**

Growth promotion test may be done in concurrent with product sterility testing.

Using 100 CFU/mL of reference microorganisms, inoculate the Thioglycollate and Soybean Casein Digest media as per Table 4.

*Table 4: Reference strains for Suitability (Growth Promotion) and Validation Tests*

<table>
<thead>
<tr>
<th>Media</th>
<th>Organisms</th>
<th>Incubation Conditions</th>
<th>Temperature</th>
<th>Suitability</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean-Casein Digest</td>
<td><em>B. subtilis</em> ATCC 6633</td>
<td>22.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em> ATCC 10231</td>
<td>22.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. brasiliensis/niger</em> ATCC 16404</td>
<td>22.5 ± 2.5°C</td>
<td>5 days</td>
<td>5 days</td>
<td></td>
</tr>
<tr>
<td>Thioglycollate fluid</td>
<td><em>C. sporogenes</em> ATCC 19404</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em> ATCC 9027</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> ATCC 6538</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
<td></td>
</tr>
</tbody>
</table>

- Soybean-Casein Digest for the culture of fungi and aerobic bacteria incubated at 22.5 ± 2.5°C
- Fluid Thioglycollate for the culture of anaerobic bacteria incubated at 30 – 35 °C
- Testing media are challenged with 10-100 CFU/mL of reference ATCC strains as per USP <71>.
- Volume of sample under test is ≤10% of media i.e. 90% medium and 10% product
IV. Reporting

Tests results are recorded on the respective log sheet and reviewed by the QA.

- Growth Promotion and Sterility Log.xls
- Validation Bacteriostasis-Fungistasis Log.xls
Validation of Suitability for Bone Bank Sterility Testing

I. Introduction

A sterility test is technically not valid unless a Suitability Test (Growth Promotion) and a Validation Test (Bacteriostasis and Fungistasis Test) are performed as per (USP) United States Pharmacopeia <61>, <62>, <71> guidelines. The Suitability test confirms that each lot of growth media used will support the growth of less than 100 viable microorganisms. The Validation test determines that the test sample does not possess any inhibiting factors to the growth of microorganisms in the test media and cause a false negative sterility test.

II. Reagents/Materials/Media

Media
In accordance with USP <61> guidelines, commercially prepared fluid Thioglycollate media (FTM) can be use for Bone Bank sterility testing. (If testing media are prepared in-house, samples must be selected from every load sterilized for testing and pH check).

Reference ATCC strains

Table 1: Reference strains for Suitability (Growth Promotion) & Validation

<table>
<thead>
<tr>
<th>Type</th>
<th>Organism</th>
<th>Reference strain</th>
<th>Incubation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 6538</td>
<td>30-35°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027</td>
<td>30-35°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 6633</td>
<td>20-25°C for 24 hours</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Candida albicans</em></td>
<td>ATCC 10231</td>
<td>20-25°C for 24 hours</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus brasiliensis/niger</em></td>
<td>ATCC 16404</td>
<td>20-25°C for 3 days</td>
</tr>
</tbody>
</table>

Sampling of Specimens
Samples for sterility testing are submitted by Bone Bank with minimum quantity of product to be tested from each container as per USP <71> table 2.

Table 2: Minimum quantity to be tested from each container

<table>
<thead>
<tr>
<th>Product type</th>
<th>Product Quantity</th>
<th>Minimum inoculum for each medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids</td>
<td>&lt;1mL</td>
<td>Whole content</td>
</tr>
<tr>
<td></td>
<td>1- 40 mL</td>
<td>Half the contents but not &lt;1mL</td>
</tr>
<tr>
<td></td>
<td>41 – 100 mL</td>
<td>20 mL</td>
</tr>
<tr>
<td></td>
<td>&gt;100 mL</td>
<td>10% contents but not &lt;20mL</td>
</tr>
</tbody>
</table>
III. Procedure

Test methods for Suitability and Validation are done by the direct transfer of the product and/or reference organisms into the fluid thioglycollate medium (FTM).

Media testing
For in-house prepared media, each sterilized load of medium must be tested for pH, sterility and growth promotion. For commercially prepared media, a certificate of growth promotion must accompany the media if the media is to be exempted from repeat testing by sterility testing laboratory.

pH Testing

Table 3: pH ranges for Thioglycollate Broth

<table>
<thead>
<tr>
<th>Medium</th>
<th>pH after sterilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioglycollate</td>
<td>7.1 ± 0.2</td>
</tr>
</tbody>
</table>

Reference ATCC strains Preparation

Reconstitute microorganisms as per Kwik-Stik manufacturer’s insert. Subculture to non-selective agar and incubate as per Table 1. Subcultured isolates are stored in trisodium citrate glycerol at -70ºC as stock cultures. Monthly, stock cultures are subcultured to non-selective agar and then to Trypticase Soy Agar slope (TSA) as working culture.

Prepare a working suspension of 100 CFU/mL of microorganism:
1. Subculture from the TSA slope to Blood agar plate and incubate as per Table 1
2. Prepare a standardized 0.5 McFarland (1 x 10^8 CFU/mL) of the 24 hours culture in 9.9 mL saline.
3. Pipette 0.1mL of the 0.5McFarland suspension into 9.9 mL saline to obtain 1:100 dilution (A) (1 x 10^6 CFU/mL)
4. Pipette 0.1mL of (A) into 9.9 mL saline to obtain 1:10,000 dilution (B) (1 x 10^4 CFU/mL)
5. Pipette 0.1mL of (B) into 0.9mL saline to obtain 1:100,000 dilution (C) (1 x 10^3 CFU/mL)
6. Dispense 0.1mL of final dilution (C) to blood agar plate and perform colony count to confirm final concentration of 100 CFU

Suitability (Growth Promotion) Testing

Growth promotion test may be done in concurrent with product sterility testing.
1. Prepare 12 Thioglycollate tubes labeled as:
Tube 1: Sterility Control
Tube 2: Sterility Control
Tube 3: S. aureus
Tube 4: S. aureus
Tube 5: P. aeruginosa
Tube 6: P. aeruginosa
Tube 7: B. subtilis
Tube 8: B. subtilis
Tube 9: C. albicans
Tube 10: C. albicans
Tube 11: A. brasiliensis/niger
Tube 12: A. brasiliensis/niger

2. Pipette 1 mL of prepared sample into each tube.
3. Pipette 0.01 mL of the 1 x 10^4 CFU/mL of reference microorganisms (Sample B from above) into the Thioglycollate media as per Table 4.

Validation (Bacteriostasis/Fungistasis) Testing

Bacteriostasis and fungistasis is performed for all new products and re-validated when there’s a change in procedure or protocol. Inoculum of 10 – 100 CFU/mL of reference organism is added directly to the testing media which contains the testing product. Test is valid if the challenge organism show visible growth in the test media containing product, within 3 days for bacteria and within 5 days for fungi. Refer to Table 4

Table 4: Reference strains for Suitability (Growth Promotion) and Validation Tests

<table>
<thead>
<tr>
<th>Media</th>
<th>Organisms</th>
<th>Incubation Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temperature</td>
</tr>
<tr>
<td>Thioglycollate Broth</td>
<td>B subtilis ATCC 6633</td>
<td>35°C</td>
</tr>
<tr>
<td></td>
<td>C albicans ATCC 10231</td>
<td>35°C</td>
</tr>
<tr>
<td></td>
<td>A brasiliensis ATCC 16404</td>
<td>35°C</td>
</tr>
<tr>
<td></td>
<td>P aeruginosa ATCC 9027</td>
<td>35°C</td>
</tr>
<tr>
<td></td>
<td>S aureus ATCC 6538</td>
<td>35°C</td>
</tr>
</tbody>
</table>

1. For each specimen, label 5 Thioglycollate Broth tubes, each with specimen number and a reference organism in Table 4.
2. Label 2 other Thioglycollate Broth tubes as negative controls.
3. Pipette 1mL of specimen into each of the 7 labelled Thioglycollate Broth tubes.
4. To the tubes labeled with organisms, add 100 µL of the $1 \times 10^4$ CFU/mL of reference microorganisms (Sample B from above).

IV. **Reporting**

Tests results are recorded on a respective log sheet.

In LIS orders, report as follows:

Environmental Culture:
- Sterility Control Sample: No growth
- Growth Control Samples:
  - *S. aureus* ATCC 6538 - Growth
  - *P. aeruginosa* ATCC 9027 - Growth
  - *B subtilis* ATCC 6633 - Growth
  - *C albicans* ATCC 10231 - Growth
  - *A brasiliensis/niger* ATCC 16404 – Growth
Validation of Suitability for Pharmacy and Cell Therapy Products Sterility Testing by BacT/Alert Dual T System

I. Introduction

A sterility test is technically not valid unless a Growth Promotion (Bacteriostasis and Fungistasis Test) and Suitability Test (Validation) are performed as per (USP) United States Pharmacopeia <71> guidelines. The Growth Promotion test confirms that each lot of growth media used will support the growth of less than 100 viable microorganisms. The Validation test determines that the test sample does not possess any inhibiting factors to the growth of microorganisms in the test media and cause a false negative sterility test with 10-100 viable microorganisms.

II. Reagents/Materials/Media

**Media**

BacT/Alert iFA Plus and iFN Plus bottles

*Reference ATCC strains with Biomerieux Bioballs*

**Table 1: Reference strains for Suitability and Growth Promotion**

<table>
<thead>
<tr>
<th>Type</th>
<th>Organism</th>
<th>Reference strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic</td>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 6538</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus subtilis</em></td>
<td>ATCC 6633</td>
</tr>
<tr>
<td>Anaerobic</td>
<td><em>Clostridium sporogenes</em></td>
<td>ATCC 19404</td>
</tr>
<tr>
<td>Fungi</td>
<td><em>Candida albicans</em></td>
<td>ATCC 10231</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus brasiliensis/niger</em></td>
<td>ATCC 16404</td>
</tr>
</tbody>
</table>

**Sampling of Specimens**

Samples for sterility testing are submitted by pharmacies or cell therapy preparation facilities with minimum quantity of product to be tested from each container as per USP <71> table 2.

*Table 2: Minimum quantity to be tested from each container*

<table>
<thead>
<tr>
<th>Product type</th>
<th>Product Quantity</th>
<th>Minimum inoculum for each medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquids</td>
<td>&lt;1mL</td>
<td>Whole content</td>
</tr>
<tr>
<td></td>
<td>1- 40 mL</td>
<td>Half the contents but not &lt;1mL</td>
</tr>
</tbody>
</table>
III. **Procedure**

Test methods for Suitability and Growth Promotion are done by the direct transfer of the product and/or reference organisms into BacT/Alert iFA Plus and iFN Plus bottle.

**Media testing**

For BacT/Alert iFA Plus and iFN Plus bottle, obtain a certificate of growth promotion for each of the media.

**Reference ATCC strains Preparation for each organism**

1. Transfer one BioBall into 1.1 mL rehydration fluid and wait for 3 minutes.
2. Vortex for 5 seconds.
3. Inoculate 0.1 mL rehydrated BioBall to appropriate media for the original colony count.
4. Inoculate 0.2 mL rehydrated BioBall into 9.8 mL 0.45% saline.
5. Mix well and inject 1 mL **organism suspension** (estimated 10 CFU) into each bottle.
6. Triplicate for each bottle type for each organism.
7. Inoculate 1 mL **organism suspension** onto appropriate solid media (TSA or TSA with 5% Sheep Blood) to confirm the **actual colony count**.
8. Aliquot 0.25 mL from the remaining 0.8 mL rehydrated BioBall into 3 Eppendorf tubes for future use and put them in -18°C for storage.
9. The aliquot Eppendorf tube is stable for ONE week when it is stored at -18°C.

**Growth Promotion Testing**

Growth promotion testing may be done in concurrent with product sterility testing. Inoculate 10-100 CFU of reference microorganisms into each BacT/Alert iFA Plus and iFN Plus bottle.

**Suitability (Validation) Testing**

Suitability testing is performed for all new products and re-validated when there’s a change in procedure or protocol. Inoculum of less than 100 CFU of reference organism is added directly.
to the testing media which contains the testing product. Test is valid if the challenge organism show visible growth in the test media containing product, within 3 days for bacteria and within 5 days for fungi.
For each product to be tested, transfer up to 10 mL of the specimen into each of bottle. To each bottle add 1 mL of the rehydrated suspension of the respective reference microorganisms. 

Refer to Table 4

Table 4: Reference strains for Suitability (Growth Promotion) and Validation Tests

<table>
<thead>
<tr>
<th>Media</th>
<th>Organisms</th>
<th>Incubation Conditions</th>
<th>Suitability</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>iFA Plus</td>
<td><em>B. subtilis</em> ATCC 6633</td>
<td>22.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td><em>C. albicans</em> ATCC 10231</td>
<td>22.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td><em>A. brasiliensis/niger</em> ATCC 16404</td>
<td>22.5 ± 2.5°C</td>
<td>5 days</td>
<td>5 days</td>
</tr>
<tr>
<td>iFN Plus</td>
<td><em>C. sporogenes</em> ATCC 19404</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginos</em> ATCC 9027</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> ATCC 6538</td>
<td>32.5 ± 2.5°C</td>
<td>3 days</td>
<td>5 days</td>
</tr>
</tbody>
</table>

a. iFA Plus for the culture of fungi and aerobic bacteria incubated at 22.5 ± 2.5°C
b. iFN Plus for the culture of anaerobic bacteria incubated at 30 – 35 °C
c. Testing media are challenged with 10-100 CFU/mL of reference ATCC strains as per USP <71>.
d. Volume of sample under test is ≤10% of media i.e. 90% medium and 10% product (see Table 2)

IV. Reporting

Tests results are recorded on the respective log sheet and reviewed by QA.

Growth Promotion and Sterility Log.xls
Validation Bacteriostatics-Fungistasis Log.xls
Record of Edited Revisions

Manual Section Name: Sterility Manual

<table>
<thead>
<tr>
<th>Page Number / Item</th>
<th>Date of Revision</th>
<th>Signature of Approval</th>
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<tbody>
<tr>
<td>Pg. 3 Environmental Specimen - incubation for 14 days for PET center added</td>
<td>16-Jan-04</td>
<td>Dr. T. Mazzulli</td>
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<td>Pg. 4 Remove PET Centre from Pharmacy Sterility</td>
<td>3-May-04</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>26-May-04</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Bone and Bone Bank Specimens - Fresh Osteochondral Allograft added</td>
<td>2-Mar-05</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>12-May-05</td>
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<td>23-Jul-06</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Spore strips – Midmark ultraclave added</td>
<td>3-May-07</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>3-May-07</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Change Pharmacy specimen incubation to 14 days</td>
<td>28-Jul-08</td>
<td>Dr. T. Mazzulli</td>
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<td>Tissue culture specimens for injection</td>
<td>28-Jul-08</td>
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<td>Annual Review</td>
<td>28-Jul-08</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Re-organized Table of Contents</td>
<td>27-Jul-09</td>
<td>Dr. T. Mazzulli</td>
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<td>Moved Contact Lens/Solution from Wounds/Tissues Manual</td>
<td>27-Jul-09</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>27-Jul-09</td>
<td>Dr. T. Mazzulli</td>
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<td>Section for Validation of sterility testing added</td>
<td>1-Apr-10</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added Materials for Radiopharmacy</td>
<td>19-May-10</td>
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<td>27-Jul-10</td>
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<td>Revised Environmental monitoring section</td>
<td>15-Jun-11</td>
<td>Dr. T. Mazzulli</td>
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<td>14-Jul-11</td>
<td>Dr. T. Mazzulli</td>
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<td>Revised Radiopharmacy section</td>
<td>14-Jul-11</td>
<td>Dr. T. Mazzulli</td>
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<td>Added missing dilution line for validation</td>
<td>14-Apr-12</td>
<td>Dr. T. Mazzulli</td>
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<td>Annual Review</td>
<td>14-Apr-12</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added testing of lot numbers for positive Radiopharmacy out of specifications investigations</td>
<td>18-Dec-12</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added testing of lot numbers for positive Manufacturing pharmacy out of specifications investigations</td>
<td>18-Dec-12</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Radiopharmacy</td>
<td>3-Jan-13</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Radiopharmacy</td>
<td>3-Jan-13</td>
<td>Dr. T. Mazzulli</td>
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<td>Date of Revision</td>
<td>Signature of Approval</td>
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<tr>
<td>-----------------------------------------------------------------------------------</td>
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<td>Manufacturing pharmacy</td>
<td></td>
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<tr>
<td>Annual Review</td>
<td>31-May-13</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Updated Environmental reading &amp; reporting</td>
<td>27-Sep-13</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>3-Jul-14</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Removed all text in all sections under specimen collection and transportation and replaced it with link to Specimen collection manual QPCMI02001 where info is now housed.</td>
<td>26-May-15</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>3-Jul-15</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Environmental samples: modified procedure to Identify any amount of organism. (including &lt;5 colonies).</td>
<td>5-May-16</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Addition of Endoscope surveillance swab section</td>
<td>28-Oct-16</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Hemodialysis Water Procedure has been updated following CSA guidelines. Media and incubation conditions have been updated. Preliminary 48hrs report added.</td>
<td>8-Dec-17</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updated Manufacturing Pharmacy section to Pharmacy Samples Generalized Radiopharmacy to Pharmacy Updated Bonebank sample instructions to include receipt of a positive control with workup and reporting.</td>
<td>7-Mar-18</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Annual Review</td>
<td>22-May-18</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Addition of Media fill to Environmental sample section Updated Environmental Air/Touch/Finger tests incubation times to match updated USP/NAPRA guidelines. Addition of Leech procedure.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clarified hemodialysis water to daily readings and prelim results</td>
<td>5-Jun-18</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updates BA and ENACT from environmental screening with 7 day protocol to routine 3 day 35C protocol.</td>
<td>20-Jul-18</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updates reporting of environmental sterility samples to include phrases for no cfu of organism isolated.</td>
<td>25-Jul-18</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Modified reporting phrases for environmental testing.</td>
<td>August 27th, 2018</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Environmental preliminary phrases added.</td>
<td>25-Jan-19</td>
<td>Dr. T. Mazzulli</td>
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<tr>
<td>Added Pharmacy and Cell Therapy Products Sterility by BacT/Alert Dual T</td>
<td>10-Jun-19</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>pg 34-35 added workflow for visual check of bottles prior to loading clarify procedure for Suitability Testing&quot; p.51</td>
<td>30-Oct-19</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Added Validation of Pharmacy and Cell Therapy Products</td>
<td>June 10, 2019</td>
<td>Dr. T. Mazzulli</td>
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<td>Sterility by BacT/Alert Dual T</td>
<td>November 26, 2019</td>
<td>Dr. T. Mazzulli</td>
</tr>
<tr>
<td>Updated name of “Pharmacy Samples” to “Product Sterility Samples”. Updated procedures for Product Sterility Samples and Pharmacy and Cell Therapy Products Sterility by BacT/Alert Dual T to include processing of positive broths within Class II dedicated BSC. Updated all sample final reports to interim reports. Updated procedure for pharmacy samples received turbid upon receipt. Updated sterility product samples to include inspection of broths for rejection criteria prior to incubation.</td>
<td>November 26, 2019</td>
<td>Dr. T. Mazzulli</td>
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