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## 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.
Culture:

| Media | Incubation |
|---|---|
| Fastidious Anaerobic Broth (THIO) | $O_2$, $35^oC$ x 7 days |

### 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:

| Media | Incubation |
|---|---|
| Chocolate Agar (CHOC) | $CO_2$, $35^oC$ x 48 hours (examine at 24 and 48 hours) |
| Brucella Agar (BRUC) | $ANO_2$, $35^oC$ x 48 hours (examine at 48 hours) |

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.
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 Osteochandral 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:

| Media | Incubation |
|---|---|
| Fastidious Anaerobic Broth (THIO) | $O_2$, $35^oC$ x 7 days |

### 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:

| Media | Incubation |
|---|---|
| Chocolate Agar (CHOC) | $CO_2$, $35^oC$ x 48 hours (examine at 24 and 48 hours) |

| | |
|---|---|
| Brucella Agar (BRUC) | $ANO_2$, 35°C x 48 hours (examine at 48 hours) |

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. |
| Positive Report: | Remove "no growth…" statement, report based on gram smear and any preliminary identification.<br>Telephone all positive reports to the Bone Bank. |

Final Report:

| | |
|---|---|
| Negative Report: | "No Growth" |
| Positive Report: | Report all isolates without quantitation.<br>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:

| Media | Incubation |
| --- | --- |
| Blood Agar (BA) | $O_2$, 35°C x 48 hours |
| MacConkey Agar (MAC) | $O_2$, 35°C x 48 hours |
| Fastidious Anaerobic Broth (THIO) | An$O_2$, 35°C x 7 days |

### 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.

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:

| **Media** | **Incubation** |
|---|---|
| Thioglycolate Broth (THIO) | $O_2$, 35°C x 48 hours |
| MacConkey Agar (MAC)* | $CO_2$, 35°C x 48 hours |
| Colistin Nalidixic Acid Agar (CNA) | $CO_2$, 35°C x 48 hours |
| Chocolate Agar (CHOC) | $CO_2$, 35°C x 48 hours |

## 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/Sulfamethoxazle 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/Sulfamethoxazle 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

| | |
| --- | --- |
| Negative Report: | "No Growth" |
| Positive Report: | Report all aerobic isolates without quantitation.<br>Report with appropriate susceptibilities. |

## References

Bauters T, Buyle F, Blot S, Robays H, Vogelaers D, Van Landuyt K, et al. Prophylactic use of levofloxacin during medicinal leech therapy. Int J Clin Pharm 2014; 36: 995-99.

Elyassi AR, Terres J, Rowshan HH. Medicinal leech therapy on head and neck patients: A review of the literature and proposed protocol. Oral Maxilofac Surg 2013; 116:e167-e172.

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Kruer RM, Barton CA, Roberti G, Gilbert B, McMillian WD. Antimicrobial prophylaxis during *Hirudo medicinalis* Therapy: A multicenter study. J Reconstr Microsurg 2015; 31:205-209.

LEECHES U.S.A. General Information [Internet]. Wetbury, New York. 2017. [cited 2017 Nov 17]. Available from: http://www.leechesusa.com/information/general-information.

Government of Canada. Good Manufacturing Practices (GMP) Guidelines - 2009 Edition version 2. Available from: http://www.hc-sc.gc.ca/dhp-mps/compli-conform/gmp-bpf/docs/gui-0001-eng.php#sterlie

# 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:

| Media | Incubation |
|---|---|
| Blood Agar (BA) | $O_2$, 35°C x 48 hours |
| MacConkey Agar (MAC) | $O_2$, 35°C x 48 hours |
| Fastidious Anaerobic Broth (THIO) | An$O_2$, 35°C x 14 days |

## 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.

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

| Type of organism | Media | Incubation |
|---|---|---|
| Bacteria | Blood Agar | 37°C x 48 hours |
| Fungi | Inhibitory Mold Agar | 30°C x 7days |
| Bacteria<br>Fungi | Trypticase Casein Agar | 37°C x 48 hours<br>30°C x 7 days |
| Fungi | Rose Bengal Agar | 30°C x 7 days |

### 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*** x ***b*** L = ***ab***/1000 $m^3$ = ***d*** $m^3$
Bacterial or mould count = ***c*** CFU
Total CFU/$m^3$ air sampled = **c**/**d** CFU/$m^3$ air

3. Identify organism only if requested.

## V. Sensitivity Testing

Not required.

## VI. Reporting

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

Lynn E. Garcia. 2007. Air Cultures for Fungi p. 13.9.1 – 13.9.7 In Clinical Microbiology Procedures Handbook, 3rd Edition, Vol 3 ASM Press, Washington, D.C

# Attest

## 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:

| | STEAM ATTEST | FLASH ATTEST | GAS ATTEST |
|---|---|---|---|
| Cap Colour | Brown | Blue | Green |
| Incubation Temp. | 56°C | 56°C | 37°C |
| Negative Colour | Purple | Purple | Green |
| Positive Colour | Yellow | Yellow | Yellow |

## IV. Reporting

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

## Control

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 $56^0$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 ($56^0$C-$65^0$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.
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:

| **Media** | **Incubation** |
|---|---|
| Blood Agar (BA) | $CO_2$, 35°C x 5 days |
| Chocolate Agar (CHOC) | $CO_2$, 35°C x 5 days |
| Fastidious Anaerobic Broth (THIO) | $O_2$, 35°C x 5 days |
| Inhibitory Mold Agar (IMA)* | $O_2$, 30°C x 3 weeks |

*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

Negative report: Preliminary Report: "No growth to date, further report to follow" Status as preliminary (^P) after every reading
Final 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 QPCMI02001

### 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$^{o}$C x 24 hrs
5. Remove the plate from the incubator and incubate the plate at room temperate for an additional 24 hours.
6. Count and record the number of colonies on the entire agar surface.

### IV. Reporting

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 $O_2$ at 35°C.

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

Culture:

| **Media** | **Incubation** |
|---|---|
| BHI Broth | $O_2$, 35°C Overnight |
| Inoculated from BHI after incubation:<br>Blood Agar (BA) | $O_2$, 35°C x 24 hours |

### 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

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 QPCMI02001

## Culture Processing

| | **Type of organism** | **Media** | **Incubation** |
|---|---|---|---|
| **Air Sampling** | Bacteria | Tryptone Soya Agar | $O_2$ 35°C x 3 days |
| **Surface Sampling** | Bacteria | Tryptone Soya Agar (with lecithin and polysorbate) - 55mm plate | $O_2$ 35°C x 3 days |
| **Gloved Fingertip Sampling** | Bacteria | Tryptone Soya Agar (with lecithin and polysorbate) | $O_2$ 35°C x 3 days |
| For High Risk Compounding **add:** | | | |
| | Fungi | Inhibitory Mold Agar | $O_2$ 30°C x 7days |

| | **Type of organism** | **Media** | **Incubation** |
|---|---|---|---|
| **Media Fill** | Bacteria | Vial with Tryptone Soya Broth | $O_2$ 35°C x 14 days |

## Interpretations

### A. Sampling plates:

| GMP Grade | Settle plates (90mm) cfu/4hrs | Contact plates (55mm) cfu/plate | Glove prints (5 fingers) cfu/glove |
|---|---|---|---|
| **A** | <1 | <1 | <1 |
| **B** | 5 | 5 | 5 |
| **C** | 50 | 25 | - |
| **D** | 100 | 50 | - |

*As per Good Manufacturing Practices (GMP) Guidelines 2009 Edition*

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.

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:

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

| | |
| --- | --- |
| Failed | "Failed - Turbid at 35C incubation temperature" |

## References

Lynn E. Garcia. 2007. Air Cultures for Fungi p. 13.9.1 – 13.9.7 In Clinical Microbiology Procedures Handbook, 3rd Edition, Vol 3 ASM Press, Washington, D.C

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

2016. Model Standards for Pharmacy Compounding of Non-Hazardous Sterile Preparations. The National Association of Pharmacy Regulatory Autorities. Ottawa, ON

# 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 7days
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"

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

## V. References

CAN/CSA-ISO 13959:15 - Water for haemodialysis and related therapies (Adopted ISO 13959:2014, third edition, 2014-04-01)

CAN/CSA-ISO 11663:15, Quality of dialysis fluid for haemodialysis and related therapies, [ISO 11663:2014, IDT], National Standard of Canada

## Miscellaneous Non-biological Samples

### 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:

| Media | Incubation |
|---|---|
| Fastidious Anaerobic Broth (THIO) | $O_2$, 35°C x 7 days<br>$O_2$, 35°C x 14 days (PET centre only) |

### 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

Negative Report: Preliminary Report: "No growth to date, further report to follow" Status as preliminary (^P) after every reading
Final Report: "No growth after 14 days."

Positive Report: Report all isolates without quantitation.

## Pharmacy Samples

### I. Introduction

Sterility testing of pharmacy products is 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 - $8^0$C

### III. Specimen Collection and Transport (by Radiopharmacy)

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 only inoculated Thioglycollate (TH14) and Tryptone Soya (SD14) broths

   **Note:** Notify and return to Radiopharmacy all Radiopharmacy products not received in Thioglycollate or Tryptone Soya broths. Only radiopharmaceutical products inoculated in Thioglycollate and Tryptone Soya broths are to be accepted for sterility testing

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

3. Incubate the tubes as below:

| **Media** | **Incubation** |
|---|---|
| **Specimens in:** | |
| Thioglycollate Broth (TH14) | $O_2$, 35°C x 14 days |
| Tryptone Soya Broth (SD14) | $O_2$, RT°C x 14 days |
| Chocolate Agar (CHOC) | $CO_2$, 35°C x 2 days |
| Fastidious Anaerobic Agar (BRUC) | $AnO_2$, 35°C x 48 hours |

## 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
Incubate media as above and exam CHOC daily for 2 days and BRUC after 48hrs incubation

All isolates are to be identified.

For all positive samples, follow Investigation of positive cultures below and report appropriately.

## VI. Sensitivity Testing

Not required

## VII. Reporting

Telephone positive report(s) to submitting pharmacy

Negative Report: Preliminary Report: "No growth to date, further report to follow" Status as preliminary (^P) after every reading
Final Report: "No growth after 14 days."

Positive Report: Report all isolates without quantitation.

## 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:**

| | Testing Facility Reference Number | Result | Media Information | |
|---|---|---|---|---|
| | | | Thioglycolate | Tryptone Soya Broth |
| **Original Sample** | | | **Lot Number:**<br>**Expiration Date:** | **Lot Number:**<br>**Expiration Date:** |

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 :**

Repeat sample and un-inoculated samples with same lot sent for testing: Yes ☐
Quarantine remaining product Yes ☐
Reviewed gloved fingertip and surface environmental testing data Yes ☐

| | | | | |
|---|---|---|---|---|
| **Repeat Sample** | | | **Lot Number:**<br>**Expiration Date:** | **Lot Number:**<br>**Expiration Date:** |
| **Uninoculated Sample** | | | | |

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

**Pharmacy Technician / Pharmacist Signature:** **Date:**

## IX. Reference

Government of Canada. Good Manufacturing Practices (GMP) Guidelines - 2009 Edition version 2. Available from: http://www.hc-sc.gc.ca/dhp-mps/compli-conform/gmp-bpf/docs/gui-0001-eng.php#sterlie

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

2016. Model Standards for Pharmacy Compounding of Non-Hazardous Sterile Preparations. The National Association of Pharmacy Regulatory Autorities. Ottawa, ON

## 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 another1-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.

| Sterilization Method | Incubation Temperature | Length of Incubation |
|---|---|---|
| Autoclave | 56°C heating block | 7 days |
| Statim autoclave | 56°C heating block | 7 days |
| Midmark Ultraclave | 56°C heating block | 7 days |
| Chemiclave | 35°C incubator | 7 days |
| Radiation (primarily from Bone Bank) | 35°C incubator | 7 days |

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.

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"

## Validation of Sterility Testing

## Validation of Suitability for Radiopharmacy 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 <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 <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*

| Type | Organism | Reference strain | Incubation Conditions |
|---|---|---|---|
| Aerobic | Staphylococcus aureus | ATCC 6538 | 30-35$^{o}$C for 24 hours |
| | Pseudomonas aeruginosa | ATCC 9027 | 30-35$^{o}$C for 24hours |

| | | | |
|---|---|---|---|
| | Bacillus subtilis | ATCC 6633 | 20-25°C for 24 hours |
| Anaerobic | Clostridium sporogenes | ATCC 19404 | 30-35°C for 48 hours |
| Fungi | Candida albicans | ATCC 10231 | 20-25°C for 24 hours |
| | Aspergillus brasiliensis/niger | ATCC 16404 | 20-25°C for 3 days |

***Sampling of lots***

Samples for sterility testing are submitted by Radiopharmacy 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*

| Product type | Product Quantity | Minimum inoculum for each medium |
| --- | --- | --- |
| Liquids | <1mL | Whole content |
| | 1- 40 mL | Half the contents but not <1mL |
| | 41 – 100 mL | 20 mL |
| | >100 mL | 10% contents but not <20mL |

**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 Validation 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*

| Medium | pH after sterilization |
| --- | --- |
| Fluid Thioglycollate | 7.1 ± 0.2 |
| Soybean-Casein Digest | 7.3 ± 0.2 |

## 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 -70$^{o}$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 $10^8$ 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.

Using 100 CFU/mL of reference microorganisms, inoculate the Thioglycollate and Soybean Casein Digest 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.
For each specimen, pipette 1ml of the specimen into each of the media. To each tube then add 100 uL of the 1 x $10^4$ CFU/mL of the respective reference microorganisms.
*Refer to Table 4*

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

| Media | Organisms | Incubation Conditions | | |
|---|---|---|---|---|
| | | Temperature | Suitability | Validation |
| Soybean-Casein Digest | B subtilis ATCC 6633 | 22.5 ± 2.5°C | 3 days | 5 days |
| | C albicans ATCC 10231 | 22.5 ± 2.5°C | 3 days | 5 days |
| | A brasiliensis/niger ATCC 16404 | 22.5 ± 2.5°C | 5 days | 5 days |
| Thioglycollate fluid | C sporogenes ATCC 19404 | 32.5 ± 2.5°C | 3 days | 5 days |
| | P aeruginosa ATCC 9027 | 32.5 ± 2.5°C | 3 days | 5 days |
| | S aureus ATCC 6538 | 32.5 ± 2.5°C | 3 days | 5 days |

- 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 $^{0}$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.
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*

| Type | Organism | Reference strain | Incubation Conditions |
|---|---|---|---|
| Aerobic | *Staphylococcus aureus* | ATCC 6538 | 30-35$^{o}$C for 24 hours |
| | *Pseudomonas aeruginosa* | ATCC 9027 | 30-35$^{o}$C for 24hours |
| | *Bacillus subtilis* | ATCC 6633 | 20-25$^{o}$C for 24 hours |
| Fungi | *Candida albicans* | ATCC 10231 | 20-25$^{o}$C for 24 hours |

| | | | |
|---|---|---|---|
| | *Aspergillus brasiliensis/niger* | ATCC 16404 | 20-25°C for 3 days |

***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*

**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 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*

| Medium | pH after sterilization |
|---|---|
| Thioglycollate | 7.1 ± 0.2 |

**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 -70$^{o}$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 $10^8$ 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*

| Media | Organisms | Incubation Conditions | | |
|---|---|---|---|---|
| | | Temperature | Suitability | Validation |
| Thioglycollate Broth | *B subtilis* ATCC 6633 | 35°C | 3 days | 5 days |

| | | | | |
|---|---|---|---|---|
| | *C albicans* ATCC 10231 | 35°C | 3 days | 5 days |
| | *A brasiliensis* ATCC 16404 | 35°C | 5 days | 5 days |
| | *P aeruginosa* ATCC 9027 | 35°C | 3 days | 5 days |
| | *S aureus* ATCC 6538 | 35°C | 3 days | 5 days |

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 x $10^4$ 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

## *Record of Edited Revisions*

**Manual Section Name: Sterility Manual**

| Page Number / Item | Date of Revision | Signature of Approval |
|---|---|---|
| Annual Review | September 25, 2002 | Dr. T. Mazzulli |
| Pg. 3 Environmental Specimen - incubation for 14 days for PET center added | January 16, 2004 | Dr. T. Mazzulli |
| Pg. 4 Remove PET Centre from Pharmacy Sterility | May 03, 2004 | Dr. T. Mazzulli |
| Annual Review | May 26, 2004 | Dr. T. Mazzulli |
| Bone and Bone Bank Specimens - Fresh Osteochandral Allograft added | March 2, 2005 | Dr. T. Mazzulli |
| Annual Review | May 12, 2005 | Dr. T. Mazzulli |
| Annual Review | July 23, 2006 | Dr. T. Mazzulli |
| Spore strips – Midmark ultraclave added | May 3, 2007 | Dr. T. Mazzulli |
| Annual Review | May 3, 2007 | Dr. T. Mazzulli |
| Change Pharmacy specimen incubation to 14 days | July 28, 2008 | Dr. T. Mazzulli |
| Tissue culture specimens for injection | July 28, 2008 | Dr. T. Mazzulli |
| Annual Review | July 28, 2008 | Dr. T. Mazzulli |
| Re-organized Table of Contents | July 27, 2009 | Dr. T. Mazzulli |
| Moved Contact Lens/Solution from Wounds/Tissues Manual | July 27, 2009 | Dr. T. Mazzulli |
| Annual Review | July 27, 2009 | Dr. T. Mazzulli |
| Section for Validation of sterility testing added | April 01, 2010 | Dr. T. Mazzulli |
| Added Materials for Radiopharmacy | May 19, 2010 | Dr. T. Mazzulli |
| Annual Review | July 27, 2010 | Dr. T. Mazzulli |
| Revised Environmental monitoring section | June 15, 2011 | Dr. T. Mazzulli |
| Annual Review | July 14, 2011 | Dr. T. Mazzulli |

| | | |
|---|---|---|
| Revised Radiopharmacy section | July 14, 2011 | Dr. T. Mazzulli |
| Added missing dilution line for validation | April 14, 2012 | Dr. T. Mazzulli |
| Annual Review | April 14, 2012 | Dr. T. Mazzulli |
| Added testing of lot numbers for positive Radiopharmacy out of specifications investigations | December 18, 2012 | Dr. T. Mazzulli |
| Added testing of lot numbers for positive Manufacturing pharmacy out of specifications investigations | December 18, 2012 | Dr. T. Mazzulli |
| Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Radiopharmacy | January 03, 2013 | Dr. T. Mazzulli |
| Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Manufacturing pharmacy | January 03, 2013 | Dr. T. Mazzulli |
| Annual Review | May 31, 2013 | Dr. T. Mazzulli |
| Updated Environmental reading & reporting | September 27, 2013 | Dr. T. Mazzulli |
| Annual Review | July 3, 2014 | Dr. T. Mazzulli |
| 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. | May 26, 2015 | Dr. T. Mazzulli |
| Annual Review | July 3, 2015 | Dr. T. Mazzulli |
| Environmental samples: modified procedure to Identify any amount of organism. (including <5 colonies). | May 5, 2016 | Dr. T. Mazzulli |
| Addition of Endoscope surveillance swab section | October 28, 2016 | Dr. T. Mazzulli |
| Pharmacy Sterility, Manufacturing Pharmacy (UHN) and Radiopharmacy standardized into one Manufacturing Pharmacy procedure. Revised investigation of positive samples procedure .<br>Environmental Air/Touch/Contact RODAC plates updated to 35C incubation. | | |
| Hemodialysis Water Procedure has been updated following CSA guidelines. | December 8, 2017 | Dr. T. Mazzulli |

| | | |
|---|---|---|
| Media and incubation conditions have been updated.<br>Preliminary 48hrs report added. | | |
| Updated Manufacturing Pharmacy section to Pharmacy Samples<br>Generalized Radiopharmacy to Pharmacy<br>Updated Bonebank sample instructions to include receipt of a positive control with workup and reporting. | March 7, 2018 | Dr. T. Mazzulli |
| Annual Review<br>Addition of Media fill to Environmental sample section<br>Updated Environmental Air/Touch/Finger tests incubation times to match updated USP/NAPRA guidelines.<br>Addition of Leech procedure. | May 22, 2018 | Dr. T. Mazzulli |
| Clarified hemodialysis water to daily readings and prelim results | June 5, 2018 | Dr. T. Mazzulli |
| Updates BA and ENACT from environmental screening with 7 day protocol to routine 3 day 35C protocol. | July 20, 2018 | Dr. T. Mazzulli |
| Updates reporting of environmental sterility samples to include phrases for no cfu of organism isolated. | July 25, 2018 | Dr. T. Mazzulli |
| Modified reporting phrases for environmental testing. | August 27th, 2018 | Dr. T. Mazzulli |