TABLE OF CONTENTS

Biobanking – Long Isolate Storage

Introduction

Biobanking refers to a large collection of biological material amassed for future purposes often related to research. Bacteriology banking includes long term frozen isolate storage in an organized and systematic manner.

Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

Processing of Specimens

1. Ensure a fresh 24 to 48 hour culture is received.
   If a culture >48hours is received, or non-sufficient quantity of culture is received for storage, subculture onto media as below:

Organism	Media	Incubation
Aerobic bacteria	CHOC	CO_2 x 18-48 hours
Anaerobic bacteria	BRUC	CO_2 x 18-48 hours
Fungi/Mold	IMA	O_2 x 1-7 days

2. Inoculate a heavy suspension of organism into storage vials (quantity as requested) and store as below:

Organism	Media	Temperature
Aerobic bacteria	Trisodium citrate glycerol media	-70°C
Anaerobic bacteria	Trisodium citrate glycerol media	-70°C
Fungi/Mold	IMA	RT 20-22C

3. From the last vial inoculated, create a purity plate (to confirm vials were not contaminated upon inoculation) on applicable media and incubate as described in step #1

4. For each vial, assign a storage location through Softstore. Vials are frozen in BIOBANK box in MIFBB

5. After incubation of purity plate, confirm morphotypes observed on agar to document purity of isolate. Identify the isolate to confirm identification of frozen isolate submitted.

   For purity plates identifying multiple isolates (contaminated)
   - remove and discard freezer vials stored
   - assess submitted culture for mixed growth (confirm by gram sweep in primary inoculum if only 1 morphotype is observed)
     - repeat procedure if confirmed submitted culture is pure
     - if mixed culture is confirmed, document in LIS, subculture to isolate organism of interest.
     - If unable to proceed or unable to isolate organism of interest, notify senior and contact client.

Reporting

Interim Reports:

A. Successful Report:

Test comment: document as follows, listing SoftStore vial locations:

"Storage location:
_____
_____
_____
"

Isolate Comment: Report isolate without quantitation.

B. Unsuccessful Report:

Test comment: "Unable to store isolate: _____"

Refer to quality assurance technologist or charge technologist for reporting for each unsuccessful sample.

Microbial Enumeration (Bioburden) Samples

Introduction

Microbial enumeration, also known as bioburden testing, is a quantitative test of bacteria, yeast and fungi that may grow under aerobic conditions using the Plate-Count method. Bioburden may be tested on biological or non-biological specimen types.

Suitability of the test method to detect microorganism in the presence of product to be tested must be established and re-confirmed if a change in testing or product may affect the outcome of the test.

Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

Processing of Specimens

1. Upon receipt, accession broth received.

2. Inspect samples for rejection criteria as defined in Specimen Rejection Criteria manual MI_SM_RJCT.
   Note: Notify and store or return (if required by client) all products not received in Tryptone Soya broths or rejected for other reasons.

3. Label the TSB tubes with corresponding LIS number.

4. Place TSB tubes on shaker for 30 minutes.

5. Transfer 2mL of broth – 1mL onto each of two Tryptone Soya Agar plate.
   Spread inoculum over the entire agar surface.

   Transfer 2mL of broth – 1mL onto each of two Inhibitory Mold Agar plates
   Spread over plate and incubate as follows:

6. Incubate the agar as per chart below.

7. Count and record the number of colonies on the entire agar surface each day.
   For TSA, record up to $\geq$250 cfu.
   For IMA, record up to $\geq$50cfu.

Send a prelim report every day of testing. Send an interim report when incubation on all plates are complete.

Media	**Incubation**	
Tryptone Soya Agar (TSA) x2	O_2,	30-35^{o}C x 5 days
Inhibitory Mold Agar (IMA) x2	O_2,	20-25^{o}C x 7 days

For "Birth Tissue" samples only, identify any amount of organism to the species level.

Isolation and Identification

Not required.

Sensitivity Testing

Not required.

Interpretation

The total aerobic microbial count (TAMC) is the average count of cfu found on both TSA plates. If colonies of fungi are detected on this medium, they are counted as part of TAMC.

The total combined yeasts/molds count (TYMC) is the averaged count of cfu on both IMA plates. If bacterial colonies are detected on this media, they are counted as part of the TYMC.

Reporting

Preliminary & Interim reporting format for "Birth Tissue":

"Birth Tissue" with No Growth:
"Total aerobic microbial count: 0 cfu/mL"
"Total yeasts and molds count: 0 cfu/mL"

"Birth Tissue" with Growth:
"Total aerobic microbial count: ____cfu/mL including list all organism isolated"
"Total yeasts and molds count: ____cfu/mL including list all organism isolated"

Preliminary & Interim reporting format for all other samples:

Total aerobic microbial count: ____cfu/mL
Total yeasts and molds count: ____cfu/mL

Reference

2019. USP Compounding Compendium. The United States Pharmacopeial Convention, Rockville, MD

Suitability Test - Microbial Enumeration (Bioburden) Samples

Introduction

Prior to commencing Microbial Enumeration (Bioburden) testing, suitability of the test method to detect microorganism in the presence of product to be tested must be established. Suitability testing must be re-confirmed if a change in testing or product may affect the outcome of the test.

Materials

Product
Tryptone Soya broth (TSB) 10mL tube*
Tryptone Soya broth agar (TSA)
Inhibitory Mould Agar (IMA)
Pipette
ATCC microorganism strain or BioBall:

- *S.aureus* ATCC6538
- *P.aeruginosa* ATCC 9027
- *B.subtilis* ATCC 6633
- *C.albicans* ATCC 10231
- *A.brasiliensis* ATCC 16404

*Microbiology will send Tryptone Soya broths to clients with a Certificate of Analysis from Oxoid. Store broth at 2-8℃.

Specimen Collection and Transport

Unless otherwise directed the followed volumes must be tested:

- 10g or 10mL of product samples from 10 containers

The amount to be tested may be reduced for samples where:

- amount per dosage unit is less than or equal to 1mg
- amount per g or mL is less than 1mg

In a sterile environment, using aseptic techniques, inoculate product into six TSB not exceeding a 1:10 ratio of samples to broth.

Processing of Specimens

1. Upon receipt, label one of each received broth as follows:
   i. Saur 6538
   ii. Paer 9027
   iii. Bsub 6633
   iv. Calb 10231
   v. Abra 16404
   vi. Prod CNRL
   vii. Neg CNRL

2. Follow BioBall Preparation Procedure for each organism to inoculate 1mL into each broth. This will result in 100cfu in 10mL of fluid.

3. Transfer 1mL of broth onto each of two TSA plate or IMA as indicated in Table 1 below. Spread inoculum over the entire agar surface.

4. Incubate the agar as per chart below.

Table 1 - Suitability of Counting Method

Media	**Microorganism**	**Total Aerobic Microbial Count (TAMC)**	**Total Yeasts and Molds Count (TYMC)**	**Expected Results**
TSB + product	*S.aureus* ATCC6538	TSA x2 30-35°C; ≤3 days		TAMC ≤ 10cfu TYMC N/A
TSB + product	*P.aeruginosa* ATCC 9027	TSA x2 30-35°C; ≤3 days		TAMC ≤ 10cfu TYMC N/A
TSB + product	*B.subtilis* ATCC 6633	TSA x2 30-35°C; ≤3 days		TAMC ≤ 10cfu TYMC N/A
TSB + product	*C.albicans* ATCC 10231	TSA x2 30-35°C; ≤5 days	IMA x2 20-25°C; ≤5 days	TAMC ≤ 10cfu TYMC ≤ 10cfu
TSB + product	*A.brasiliensis* ATCC 16404	TSA x2 30-35°C; ≤5 days	IMA x2 20-25°C; ≤5 days	TAMC ≤ 10cfu TYMC ≤ 10cfu
TSB + product	N/A	TSA 30-35°C; 5 days	IMA 20-25°C; 5 days	TAMC 0 cfu TYMC 0cfu
TSB	N/A	TSA 30-35°C; 5 days	IMA 20-25°C; 5 days	TAMC 0 cfu TYMC 0cfu

Isolation and Identification

Not applicable.

Sensitivity Testing

Not applicable.

Interpretation

The total aerobic microbial count (TAMC) is the average count of cfu found on both TSA plates. If colonies of fungi are detected on this medium, they are counted as part of TAMC.

The total combined yeasts/molds count (TYMC) is the averaged count of cfu on both IMA plates. If bacterial colonies are detected on this media, they are counted as part of the TYMC.

A successful suitability test is determined by meeting the expected criterial in Table 1.

Reporting

A final report will be generated

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 mixed cultures, ensure to subculture isolates onto Chocolate Agar and Brucella Agar only. If subculture to other media is necessary for isolate identification, you must perform QC for the media type, document the QC and notify the QA technologist/designate before adding the media plate.

* Prepare Gram stain smear and subculture all turbid broths onto Chocolate (CHOC) and Fastidious Anaerobic (BRUC) media within the designated Class II BSC.

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

V. Sensitivity Testing

Not required.

VI. Reporting

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 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^oC x 48 hours (examine at 48 hours)

* For mixed cultures, ensure to subculture isolates onto Chocolate Agar and Brucella Agar only. If subculture to other media is necessary for isolate identification, you must perform QC

for the media type, document the QC and notify the QA technologist/designate before adding the media plate.

* Prepare Gram stain smear and subculture all turbid broths onto Chocolate (CHOC) and Fastidious Anaerobic (BRUC) media within the designated Class II BSC. 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. Telephone all positive reports to the Bone Bank.

Interim Report:

Negative Report:	"No Growth"
Positive Report:	Report all isolates without quantitation. 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)	AnO_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.

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:

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 Aeromonas isolates are to be identified, tested for susceptibilities and frozen. Consult charge/microbiologist if pure growth of other organism is detected.

Sensitivity Testing

Refer to Susceptibility Testing Manual

Reporting

Preliminary Report:

Negative Report:	"No growth to date, further report to follow"
Positive Report:	Remove "no growth…" statement, report based on gram smear and any preliminary identification.

Interim Report:

Negative Report:	"No Growth" or "No Aeromonas isolated"
Positive Report:	Report all Aeromonas isolates without quantitation. 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.

Giltner Cl, Bobenchik AM, Uslan DZ, Deville JG, Humphries RM. Ciprofloxacin-resistant *Aeromonas hydrophila* cellulitis following leech therapy. J Clin Microbiol 2013; 51(4): 1324-26.

Herline C, Bertheuil N, Bekara F, Boissiere F, Sinna R, Chaput B. Leech therapy in flap salvage: Systematic review and practical recommendations. Annales de chirurgie plastique esthétique 2017; 62:e1-e13.

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)	AnO_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.
Preliminary Report:
Negative Report: "No growth to date, further report to follow"
Positive Report: Remove "no growth…" statement, report based on gram smear and any preliminary identification.
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:

Type of organism	Media	Incubation
Bacteria	Blood Agar	37°C x 48 hours
Fungi	Inhibitory Mold Agar	30°C x 7days
Bacteria Fungi	Trypticase Casein Agar	37°C x 48 hours 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

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

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.

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

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

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 QPCMI02001

III. Processing of Specimens

1. Process sample within one hour of sampling or refrigerate at 2-8 ^{o}C up to 24 hours.
2. Samples should be gently and thoroughly mixed prior to processing.
3. Inoculate 1 mL of sample onto a R2A Agar plate and spread the inoculum over the entire agar surface with “hockey stick” and let it dry.
4. Invert the plate to prevent agar surface condensation after
5. Incubate the R2A plate at 20-28 ^{o}C for 5 days.
6. Count and record the number of colonies (up to 100) on the entire agar surface each day.
7. Send a prelim report each day the count is <100.
8. Send an interim report on day 5 or once >100 colonies have been recorded.

IV. Reporting

Interim Reports:

Negative Report:	"No Growth"
Positive Report:	Report the number of colonies recorded as “x CFU/mL”
	Or if >100 colonies counted, report “>100 x CFU/mL”

Reference: CLSI (C3-A4) Vol.26 NO.22. Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline-Forth Edition)

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^{o}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^{o}C Overnight
Inoculated from BHI after incubation: Blood Agar (BA)	O_2, 35^{o}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

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 QPCMI02001

Culture Processing

Procedure	**Type of organism**	**Media**	**Incubation (at Dual Temperatures)**
Air Sampling	Bacteria	Tryptone Soya Agar	First: O_2 30°C-35°C X 2 days; Then: RT 20°C-25°C X additional 5 days
Surface Sampling	Bacteria	Tryptone Soya Agar (with lecithin and polysorbate)- 55mm plate	First: O_2 30°C-35°C X 2 days; Then RT 20°C-25°C X additional 5 days
Gloved Fingertip Sampling	Bacteria	Tryptone Soya Agar (with lecithin and polysorbate)	First: O_2 30°C-35°C X 2 days; Then RT 20°C-25°C X additional 5 days
For High Risk Compounding **add:**			
	Fungi	Inhibitory Mold Agar	O_2 30°C x 7 days

Procedure	**Type of organism**	**Media**	**Incubation (at Dual Temperatures)**
Media Fill	Bacteria	Vial/**Bag** with Tryptone Soya Broth	First: O_2 30°C - 35°C X 7 days; Then RT 20°C - 25°C X additional 7 days

*** Incubate in O_2 (30°C - 35°C) first, followed by RT incubation (20°C-25°C). Incubating at the lower temperature first may compromise the recovery of Gram-positive cocci that are important because they are often associated with humans.**

* For mixed cultures, ensure to subculture isolates onto Chocolate Agar and Brucella Agar only. If subculture to other media is necessary for isolate identification, you must perform QC for the media type, document the QC and notify the QA technologist/designate before adding the media plate.

* All plates must be handled within the designated Class II BSC when performing daily review of media plates and/or sub culture.

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	-

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, 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 is ## CFU including list all organism isolated."

Update result daily as necessary.

ii) Interim Report:

Plates with growth:

"Total bacterial colony count is ## CFU including list all organism isolated."
"Total mould colony count is ## CFU including list all organism isolated."

Plates with No Growth:
"Total bacterial colony count is 0 CFU"
"Total mould colony count is 0 CFU"

B. Media Fill:

Interim Report:

Turbidity Not Seen: "*Passed - Sterile after 14 days incubation under dual temperature conditions*"

Turbidity Seen: "*Failed – Visible turbidity on or before 14 days incubation under dual temperature conditions*"

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

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

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
United States Pharmacopeial Convention (USP). USP general chapter <797> Pharmaceutical Compounding-Sterile Preparations. USP−NF 2021. Rockville, MD: USP; 2021.

United States Pharmacopeial Convention (USP). USP general chapter <1161> Microbiology Control and Monitoring of Aseptic Processing Environments. USP−NF 2021. Rockville, MD: USP; 2021.

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) for up to 7days
7. Count and record the number of colonies (up to 100) on the entire agar surface each day.
8. Send a prelim report each day if the count is <100.
9. Send an interim report on day 7 or once >100 colonies have been recorded.

IV. Reporting

Preliminary Report: "~No growth to date, further report to follow"
"Microbial Count: _____ CFU/mL"

Interim Reports:

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

Or if >100 colonies counted, report
"Microbial Count: > 100 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
ISO 23500-5:2019, Preparation and quality management of fluids for haemodialysis and related therapies Part 5: Quality of dialysis fluid for haemodialysis and related therapies

Hemodialysis Ultrapure Dialysate Fluid Sterility

I. Introduction

Accurate microbiological surveillance is important in the indication of microbial content of dialysis water and fluid. Ultrapure dialysis fluid shall contain a total viable microbial count of less than 0.05 CFU/mL tested through total viable microbial counts using membrane filtration techniques.
Using this technique, the complete volume of fluid, up to 1000mL is filtered through a sterile filter. Bacteria that are present in the sample are trapped on the filter which is placed face up on the surface of a nutrient media and incubated. The nutrients from the agar diffuse through the filter and allow the growth of viable bacteria as countable colonies on the agar.

I. Specimen Collection and Transport

See Pre-analytical Procedure – Specimen Collection QPCMI02001

II. Processing of Specimens

1. Note the collection time of the sample. Fluid samples should be processed as soon as possible after collection.
   - If samples cannot be analysed within 4 hours of collection, they should be stored at <10°C without freezing.
   - Avoid storage for more than 24 hours.

2. Set up the membrane filtration device in the dedicated clean BSC.
   i. Move manifold with pump and discard container into the dedicated sterility BSC.
   ii. Ensure required maintenance is completed and complete pending items prior to processing samples. *Refer to Microsart Manifold Manual*
   iii. Ensure minisart PTFE 0.20um is secured on the manifold

iv. Have ready per sample:
- ~100ml of Fluid A in a sterile conical tube
- ~50ml of 70% isopropyl alcohol or ethanol in a sterile conical tube
- ~50ml of clean water in a sterile conical tube
- Sterile forceps
- Packaged Microsart 0.45 filter cup unit
- Labelled R2A media

3. Swirl sample for 10 seconds.

4. Pour up to 100 mL of water sample at a time into the sterile filter unit with the vacuum off and replace lid of cup.

5. Start the vacuum pump, move the manifold control to the on position to pull the sample through the filter. Once done, move the manifold control to off.

6. Rinse the filter housing by adding approximately 100 mL sterile Fluid A to the unit. Turn the manifold control back on to briefly to pull the peptone rinse through the filter. Turn the control back off.

7. With sterile forceps ready, remove the cup from the base. Using aseptic techniques, carefully lift the membrane out of the filter unit, holding onto the edge of the membrane.

8. Place the membrane (face up so you can see the grid)) onto the surface of a R2A plate by gently rolling the filter membrane onto the surface of the media to avoid the formation of air pockets between the filter and the surface of the growth medium.

9. Replace the cup. Pour ~50ml of 70% isopropyl alcohol or 70% ethanol into the cup. Turn the manifold control to the on position to pull the fluid through.

10. When the fluid is through, turn the control to the off position. Pour ~50ml clean water into the cup. Turn the manifold control to the on position to pull the fluid through.

11. Once all water has passed through, turn the manifold control off and turn off the vacuum pump.

12. Remove the full cup portion and discard.

13. Remove the stopper on the right end of the manifold. Tilt the manifold to drain out any remaining water.

14. Store manifold in a clean dry place.

15. Incubate the R2A plate at room temperature (17-23°C) for up to 7days

---

16. Count and record the number of colonies (up to 100) on the entire agar surface each day.

17. Send a prelim report each day the count is <100.

18. Send an interim report on day 7 or once >100 colonies have been recorded.

IV. Reporting

Preliminary Report: "~No growth to date, further report to follow"
"Microbial Count: _____ CFU"

Interim Reports:

Negative Report:	"No Growth"
Positive Report:	Report the number of colonies recorded as "Microbial Count: _____ CFU" Or if >100 colonies counted, report "Microbial Count: > 100 CFU"

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
ISO 23500-5:2019, Preparation and quality management of fluids for haemodialysis and related therapies Part 5: Quality of dialysis fluid for haemodialysis and related therapies

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 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

Preliminary Reports:

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.

Interim Report:

Negative Report: "No growth after 14 days."
Positive Report: Report all isolates without quantitation.

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 - 8^0C

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 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.
If products are not inoculated in Thioglycollate and Tryptone Soya broths, leave samples/products at room temperature until the next business day for communication to submitter if staff on receiving bench cannot reach the submitter during after-hours, weekends or holidays.

4. Place the Thioglycollate and Tryptone Soya broths into the sterility incubators immediately after processing and incubate them 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

V. Isolation and Identification

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

For suspect growth/turbid broths:
Any work-up of primary specimens must be done within the designated Class II BSC.

Prepare Gram stain Smear of broth and subculture all turbid broths onto ONLY Chocolate (CHOC) and Fastidious Anaerobic (BRUC) media. Incubate media as below.

Media	**Incubation**
Chocolate Agar (CHOC)	CO_2, 35°C x 2 days
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 2 days

Examine CHOC daily and BRUC after 48hrs incubation documenting in LIS.
All isolates are to be identified. Note colonial morphology as applicable.

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. In the dedicated BSC, transfer a portion (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.

For broths received visually clear but with questionable growth during incubation:

Where questionable growth from broth TH14 and/or SD14 were observed at some point during incubation but with no growth on the CHOC & BRUC plates subcultured, perform a terminal subculture of broth media to CHOC & BRUC plates to confirm results at the end of the 14 day incubation period.

VI. Sensitivity Testing

Not required

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
Positive Report:	Remove "no growth…" statement, report based on gram smear and any preliminary identification without quantitation.

Final Report:

Negative Report:	Final Report: "No growth after 14 day incubation."
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:** **Expiration Date:**	**Lot Number:** **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:** **Expiration Date:**	**Lot Number:** **Expiration Date:**
Uninoculated Sample				

Product Compliant: Yes ☐ Release product
No ☐ Action:

Pharmacist / Authorized Personnel **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 Authorities. Ottawa, ON

Rapid Products Sterility Testing by BacT/Alert Dual T System

I. Introduction

Sterility testing of sampling inclusive 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 *i*FA Plus and *i*FN 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 *i*FA Plus and *i*FN 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 *i*FA Plus and *i*FN 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:

Media	Incubation	
*i*FA Plus (Aerobic Bottle)	BacT/Alert 3D	20-25^{o}C x 7 days
*i*FN Plus (Anaerobic Bottle)	BacT/Alert 3D	30-35^{o}C x 7 days

Isolation and Identification

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

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

Media	Incubation
Chocolate Agar (CHOC)	CO_2, 35°C x 2 days
Fastidious Anaerobic Agar (BRUC)	AnO_2, 35°C x 48 hours

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

* For mixed cultures, ensure to subculture isolates onto Chocolate Agar and/or Brucella Agar only. If subculture to other media is necessary for isolate identification, you must perform QC for the media type, document the QC and notify the QA technologist/designate before adding the media plate.

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
Positive Report: Remove "no growth…" statement, report based on gram smear and any preliminary identification without quantitation.

Interim Report:

Negative Report: "No growth after 7 days."
Positive Report: Report all isolates without quantitation.

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:

	Testing Facility Reference Number	**Result**	**Media Information**	
			***i*FA Plus**	***i*FN Plus**
Original Sample			**Lot Number:** **Expiration Date:**	**Lot Number:** **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:**	**Lot Number:**
Uninoculated Sample			**Expiration Date:**	**Expiration Date:**

Product Compliant: Yes ☐ Release product
No ☐ Action:

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

X. 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 Authorities. 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.

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"

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

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
Anaerobic	*Clostridium sporogenes*	ATCC 19404	30-35^{o}C for 48 hours
Fungi	*Candida albicans*	ATCC 10231	20-25^{o}C for 24 hours
	Aspergillus brasiliensis/niger	ATCC 16404	20-25^{o}C for 3 days

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

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 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

Commercially prepared media utilized requires a certificate of growth promotion to accompany the media if the media and from a verified vendor if 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 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 (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 10^4 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

Media	**Organisms**	**Incubation Conditions**		
		Temperature	**Suitability**	**Validation**
Soybean-Casein Digest	*B. subtilis* ATCC 6633	22.5 ± 2.5^{0}C	3 days	5 days
	C. albicans ATCC 10231	22.5 ± 2.5^{0}C	3 days	5 days
	A. brasiliensis/niger ATCC 16404	22.5 ± 2.5^{0}C	5 days	5 days
Thioglycollate fluid	*C. sporogenes* ATCC 19404	32.5 ± 2.5^{0}C	3 days	5 days
	P. aeruginosa ATCC 9027	32.5 ± 2.5^{0}C	3 days	5 days
	S. aureus ATCC 6538	32.5 ± 2.5^{0}C	3 days	5 days

- Soybean-Casein Digest for the culture of fungi and aerobic bacteria incubated at 22.5 ± 2.5^{0}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 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

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^{o}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

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).

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) Suitability 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

Validation of Suitability for Rapid Product 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 less than 100 viable microorganisms.

II. Reagents/Materials/Media

Media
BacT/Alert *i*FA Plus and *i*FN Plus bottles

Reference ATCC strains with Biomerieux Bioballs
Table 1: Reference strains for Suitability and Growth Promotion

Type	Organism	Reference strain
Aerobic	*Staphylococcus aureus*	ATCC 6538
	Pseudomonas aeruginosa	ATCC 9027
	Bacillus subtilis	ATCC 6633
Anaerobic	*Clostridium sporogenes*	ATCC 19404
Fungi	*Candida albicans*	ATCC 10231
	Aspergillus brasiliensis/niger	ATCC 16404

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

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 Growth Promotion are done by the direct transfer of the product and/or reference organisms into BacT/Alert *i*FA Plus and *i*FN Plus bottle

Media testing

For BacT/Alert *i*FA Plus and *i*FN 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.
9. Store the tubes upright within 2 hours of rehydration in a freezer at -18^{o}C or less.
10. The shelf life of aliquot Eppendorf tube is stable for ONE week when it is frozen at -18^{o}C or less.

Note: Cultures passes are not required or performed with bioball solutions.

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 *i*FA Plus and *i*FN 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

Media	**Organisms**	**Incubation Conditions**		
		Temperature	**Suitability**	**Validation**
*i*FA Plus	*B. subtilis* ATCC 6633	22.5 ± 2.5^{0}C	3 days	5 days
	C. albicans ATCC 10231	22.5 ± 2.5^{0}C	3 days	5 days
	A. brasiliensis/niger ATCC 16404	22.5 ± 2.5^{0}C	5 days	5 days
***i*FA Plus**	***P. aeruginosa* ATCC 9027**	**32.5 ± 2.5^{0}C**	**3 days**	**5 days**
*i*FN Plus	*C. sporogenes* ATCC 19404	32.5 ± 2.5^{0}C	3 days	5 days
	S. aureus ATCC 6538	32.5 ± 2.5^{0}C	3 days	5 days

a. *i*FA Plus for the culture of fungi and aerobic bacteria incubated at 22.5 ± 2.5^{0}C
b. *i*FA Plus for the culture of *Pseudomonas aeruginosa* incubated at 30 – 35 ^{0}C
c. *i*FN Plus for the culture of anaerobic bacteria incubated at 30 – 35 ^{0}C
d. Testing media are challenged with 10-100 CFU/mL of reference ATCC strains as per USP <71>.
e. 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 Bacteriostasis-Fungistasis Log.xls

Record of Edited Revisions

Manual Section Name: Sterility Manual

Page Number / Item	Date of Revision	Signature of Approval
Pg. 3 Environmental Specimen - incubation for 14 days for PET center added	16-Jan-04	Dr. T. Mazzulli
Pg. 4 Remove PET Centre from Pharmacy Sterility	3-May-04	Dr. T. Mazzulli
Annual Review	26-May-04	Dr. T. Mazzulli
Bone and Bone Bank Specimens - Fresh Osteochandral Allograft added	2-Mar-05	Dr. T. Mazzulli
Annual Review	12-May-05	Dr. T. Mazzulli
Annual Review	23-Jul-06	Dr. T. Mazzulli
Spore strips – Midmark ultraclave added	3-May-07	Dr. T. Mazzulli
Annual Review	3-May-07	Dr. T. Mazzulli
Change Pharmacy specimen incubation to 14 days	28-Jul-08	Dr. T. Mazzulli
Tissue culture specimens for injection	28-Jul-08	Dr. T. Mazzulli
Annual Review	28-Jul-08	Dr. T. Mazzulli
Re-organized Table of Contents	27-Jul-09	Dr. T. Mazzulli
Moved Contact Lens/Solution from Wounds/Tissues Manual	27-Jul-09	Dr. T. Mazzulli
Annual Review	27-Jul-09	Dr. T. Mazzulli
Section for Validation of sterility testing added	1-Apr-10	Dr. T. Mazzulli
Added Materials for Radiopharmacy	19-May-10	Dr. T. Mazzulli
Annual Review	27-Jul-10	Dr. T. Mazzulli
Revised Environmental monitoring section	15-Jun-11	Dr. T. Mazzulli
Annual Review	14-Jul-11	Dr. T. Mazzulli
Revised Radiopharmacy section	14-Jul-11	Dr. T. Mazzulli
Added missing dilution line for validation	14-Apr-12	Dr. T. Mazzulli
Annual Review	14-Apr-12	Dr. T. Mazzulli
Added testing of lot numbers for positive Radiopharmacy out of specifications investigations	18-Dec-12	Dr. T. Mazzulli
Added testing of lot numbers for positive Manufacturing pharmacy out of specifications investigations	18-Dec-12	Dr. T. Mazzulli
Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Radiopharmacy	3-Jan-13	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Updated investigations of positive section for repeat testing with respect to lot numbers and negative controls for Manufacturing pharmacy	3-Jan-13	Dr. T. Mazzulli
Annual Review	31-May-13	Dr. T. Mazzulli
Updated Environmental reading & reporting	27-Sep-13	Dr. T. Mazzulli
Annual Review	3-Jul-14	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.	26-May-15	Dr. T. Mazzulli
Annual Review	3-Jul-15	Dr. T. Mazzulli
Annual Review Environmental samples: modified procedure to Identify any amount of organism. (including <5 colonies).	5-May-16	Dr. T. Mazzulli
Addition of Endoscope surveillance swab section	28-Oct-16	Dr. T. Mazzulli
Annual Review Hemodialysis Water Procedure has been updated following CSA guidelines. Media and incubation conditions have been updated. Preliminary 48hrs report added.	8-Dec-17	Dr. T. Mazzulli
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.	7-Mar-18	Dr. T. Mazzulli
Annual Review 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.	22-May-18	Dr. T. Mazzulli
Clarified hemodialysis water to daily readings and prelim results	5-Jun-18	Dr. T. Mazzulli
Updates BA and ENACT from environmental screening with 7 day protocol to routine 3 day 35C protocol.	20-Jul-18	Dr. T. Mazzulli
Updates reporting of environmental sterility samples to include phrases for no cfu of organism isolated.	25-Jul-18	Dr. T. Mazzulli
Modified reporting phrases for environmental testing.	27-Aug-18	Dr. T. Mazzulli
Environmental preliminary phrases added.	January 25, 2019	Dr. T Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Annual Review Added Pharmacy and Cell Therapy Products Sterility by BacT/Alert Dual T	June 10, 2019	Dr. T. Mazzulli
Added Validation of Pharmacy and Cell Therapy Products Sterility by BacT/Alert Dual T	June 10, 2019	Dr. T. Mazzulli
pg 34-35 added workflow for visual check of bottles prior to loading clarify procedure for Suitability Testing" p.51	October 30, 2019	Dr. T. Mazzulli
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.	November 26, 2019	Dr. T. Mazzulli
No need to phone bonebank samples that are not fresh bone.	January 15, 2020	Dr. T. Mazzulli
Distilled/De-Ionized Water Sterility-processing of specimen: BHI 35oC x 24 hrs is changed to R2A 20-28 oC for 5 days	March 20,2020	Dr. T. Mazzulli
Addition of Microbial Enumeration testing	April 7, 2020	Dr. T. Mazzulli
Annual Review Minor editing on Table of Contents Minor editing for Bioball preparation instructions	August 12, 2020	Dr. T. Mazzulli

Full document review included in all updates. Bi-annual review conducted when no revision had been made within 2 years.

Page Number / Item	Date of Revision	Edited by:
Addition of the following comments for Bone Bank, Environmental samples, product sterility samples: * For mixed cultures, ensure to subculture isolates onto Chocolate Agar and/or Brucella Agar only. If subculture	December 29, 2020	Jessica Bourke

Page Number / Item	Date of Revision	Edited by:
to other media is necessary for isolate identification, you must perform QC for the media type, document the QC and notify the QA technologist/designate before adding the media plate. * Prepare Gram stain smear and subculture all turbid broths onto Chocolate (CHOC) and Fastidious Anaerobic (BRUC) media within the designated Class II BSC. Removed subculturing onto Blood Agar (SUBBA), CO_2, 35°C x 2 days, MacConkey (SUBMC) O_2, 35°C x 2 days from the Rapid Products Sterility Testing by BacT/Alert Dual T System.		
Addition of Biobanking procedure	January 13, 2021	Jessica Bourke
Updated leech workup – ID/AST on Aeromonas only	Feb 12, 2021	Wayne Chiu
Minor formatting change Addition of colonial description in products sterility testing Added procedure requirement of "For broths received visually clear but with questionable growth in the middle of incubation" in product sterility samples testing	April 11, 2021	Jessica Bourke
Removed temp from reporting of environmental monitoring	May 21, 2021	Wayne Chiu
Updated deionized/distilled water procedure and Hemodialysis water procedure. Can interim once count reaches >100	July 19, 2021	Wayne Chiu
Added biobank freezer information	December 15, 2021	Wayne Chiu
Update of incubation conditions to two temperatures goes live for environmental monitoring (Air Sampling, Surface Sampling, Gloved Fingertip Sampling and Media Fill) in accordance to USP 2021	February 28, 2022	Oliver Li
Minor formatting change	March 9, 2022	Oliver Li
Updated Rapid sterility suitability to have Pseudo in iFA bottle considering recommended BacT/alert and USP guidelines.	May 17, 2022	Jessica Bourke
Updated the signature title to Pharmacist / Authorized	July 19, 2022	Oliver Li

Page Number / Item	Date of Revision	Edited by:
Personnel in the Investigation of out of Specification Results (page 42)		
Fixed spacing of pages	November 4, 2022	Jessica Bourke
Minor formatting change	November 7, 2022	Oliver Li
In the section "*IV. Processing of Specimens*" of "*Product Sterility Samples*" procedure, added detailed instructions if products are not inoculated in THIO and TSB when received in microbiology	February 20, 2023	Oliver Li
"Environmental Monitoring" Section: Minor Formatting changes Updated the media for media fill as Vial/**Bag** with Tryptone Soya Broth (with **Bag** added)	March 01, 2023	Oliver Li
Addition of Ultrapure dialysate testing	May 5th, 2023	Jessica Bourke
Updated the reporting phrase for "Hemodialysis Water Sterility" and "Hemodialysis Ultrapure Dialysate Fluid Sterility" as per ISO 23500-5: 2019	February 20, 2024	Oliver Li
In "Microbial Enumeration (Bioburden) Samples" section, added isolation and reporting instructions for birth tissue samples.	May 23, 2024	Oliver Li