STERILITY TESTING MANUAL

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BONE and BONE BANK SPECIMENS

I. Introduction

Bone specimens and swabs from Bone Bank are submitted for sterility check. These specimens are cultured for 7 days before a final report is issued.

II. Specimen Collection and Transport

Collect specimens aseptically in sterile containers or transport it in its original container.

III. Processing of Specimens

Inoculate up to 1 mL of 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.

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)

Identify all isolates. **Freeze all isolates**.

V. Sensitivity Testing

Not required.

VI. Reporting

Telephone all positive results.
Negative Report: "No Growth"
Positive Report: Report all isolates without quantitation.

VII. Reference

American Association of Tissue Banking Standards

BONE and 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

Collect the specimen aseptically in sterile containers or transport it in its original container. Label the specimen with Last Name "BONE FRESH" and place a red dot sticker sheet inside the specimen bag. Bone Bank technologist will e-mail the Microbiology charge technologist to alert the Microbiology lab of the arrival of the specimen.

III. Processing of Specimens

Inoculate up to 1 mL of 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°C 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. **Freeze 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.

Final 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

MISCELLANEOUS STERILITY SPECIMENS

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

Collect specimen aseptically in sterile containers or transport it in its original container.

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

Gram stain and subculture all turbid Fastidious Anaerobic Broths. All isolates require minimal identification eg. *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: "No Growth"
Positive Report: Report all isolates without quantitation.

PHARMACY STERILITY

I. Introduction

Pharmacy Sterility testing is limited mainly to Pharmacy and Nuclear Medicine liquid preparations for patients. These specimens are tested for 48 hours.

II. Specimen Collection and Transport

Specimens must be collected aseptically in sterile containers or transported in its original container.

III. Processing of Specimens

Inoculate up to 1 mL of specimen into a Fastidious Anaerobic Broth. Read cultures daily for 2 days.
Culture:

Media	Incubation
Fastidious Anaerobic Broth (THIO)	O_2, 35°C x 48 hours

IV. Isolation and Identification

Gram stain and subculture all turbid Fastidious Anaerobic Broths. All isolates are to be identified.

V. Sensitivity Testing

Not required.

VI. Reporting

Telephone all positive reports
Negative Report: "No Growth"
Positive Report: Report all isolates.

RADIOPHARMACY / MANUFACTURING PHARMACY (UHN GENERAL DIVISION)

I. Introduction

Sterility testing for UHN Radiopharmacy and Manufacturing Pharmacy products is performed to ensure proper safety of all products prior to use in patients.

II. Specimen Collection and Transport

Specimens must be collected transported in its original bottle/vial or bag.

III. Processing of Specimens

Inoculate 1 mL of specimen from bottle/vial into the broths listed below.
Inoculate 10 mL of specimen from bag into the broths listed below.
Read these cultures daily for 14 days.

Culture:

Media	**Incubation**
Specimens in Bottles/Vials:	
Single strength Thioglycollate (TH14)	O_2, 35°C x 14 days
Single Strength Soya Casein Digest Broth (SD14)	O_2, RT°C x 14 days
Specimens in Bags:	
Double strength Thioglycollate (ETH14)	O_2, 35°C x 14 days
Double Strength Soya Casein Digest Broth (ESD14)	O_2, RT°C x 14 days

Note: If specimen contains Benzalkonium Chloride or any other disinfectant, inoculate 0.1 ml. of specimen on a Letheen agar plate to neutralize the disinfectant. Incubate Letheen plate for two days at 37^0C and two days at room temperature. Discard after four days.

Keep original specimen at 4°C specimen refrigerator for 1 month.

IV. Isolation and Identification

Gram stain and subculture all turbid Broths. All isolates are to be identified. Depending on results of gram stain, subculture broths onto Blood agar, MacConkey agar and any additional plates indicated.

Replant the original specimen onto a new set of media. Read the newly inoculated media for 14 days.

V. Sensitivity Testing

Not required.

VI. Reporting

Telephone all positive reports

Negative Report: "No Growth"
Positive Report: Report all isolates.

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

Specimens are sent in a clean sterile container or in their original container.

III. Processing of Specimens

i) Direct examination: Gram stain

ii) Culture:

Media	Incubation
Blood Agar (BA)	O_2, 35^0C x 48 hours
MacConkey Agar (MAC)	O_2, 35^0C x 48 hours
Fastidious Anaerobic Broth (THIO)	AnO_2, 35^0C x 7 days

IV. Isolation and Identification

All isolates are to be identified. Gram stain and subculture all turbid FAB.

V. Sensitivity Testing

Not regquired.

VI. Reporting

Telephone all positive reports to ward / physician.

Negative Report: "No Growth"
Positive Report: Report all isolates

DOG (CARDIOVASCULAR LAB) SPECIMENS

I. Introduction

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

II. Specimen Collection and Transport

Specimens are sent in a clean sterile container or in their original container.

III. Processing of Specimens

iii) Direct examination: Gram stain

iv) Culture:

Media	Incubation
Blood Agar (BA)	O_2, 35^0C x 48 hours
MacConkey Agar (MAC)	O_2, 35^0C x 48 hours
Fastidious Anaerobic Broth (THIO)	AnO_2, 35^0C x 7 days

IV. Isolation and Identification

All isolates are to be identified. Gram stain and subculture all turbid FAB.

V. Sensitivity Testing

Not required.

VI. Reporting

Telephone all positive reports to ward / physician.

Negative Report: "No Growth"
Positive Report: Report all isolates

ATTEST

I. Introduction

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

II. Specimen Collection and Transport

The media-containing glass ampoule must be intact until activated in the lab. Damaged Attests will be rejected.

III. Procedure

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

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

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

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

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

	STEAM ATTEST	FLASH ATTEST	GAS ATTEST
Cap Colour	Brown	Blue	Green
Incubation Temp.	56^0C	56^0C	37^0C
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. 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.

III. 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"

TML/MSH Microbiology Department Policy & Procedure Manual	**Policy # MI\STER\09\v01**	Page 1 of 1
Section: **Sterility Testing Manual**	Subject Title: **Distilled/De-ionised Water Sterility**	
Issued by: **LABORATORY MANAGER**	Original Date: July 17, 2001	
Approved by: Laboratory Director	Revision Date:	

DISTILLED/DE-IONISED WATER STERILITY

I. Introduction

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

II. Specimen Collection and Transport

1. Open the water tap fully and allow the water to run for a minimum of 1 minute before sampling.
2. Collect a minimum of 10 mL of water into a sterile container large enough to hold the entire sample with ample of air space to allow for mixing. Avoid any splashing.

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”

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. The spore strip is then sent to the lab for testing after the sterilization process. A control strip (unsterilized) may be sent along for testing.

II. 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^{o}C heating block	7 days
Statim autoclave	56^{o}C heating block	7 days
Chemiclave	35^{o}C incubator	7 days
Radiation (primarily from Bone Bank)	35^{o}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.

III. 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"

AIR SAMPLING

II. 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 apparati 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. Procedure

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

Type of organism	Media	**Incubation**
Bacteria	**Blood Agar**	37^{o}C x 48 hours
Fungi	Inhibitory Mold Agar	30^{o}C x 7days
Bacteria Fungi	Trypticase Casein Agar	37^{o}C x 48 hours 30^{o}C x 7 days
Fungi	Rose Bengal Agar	30^{o}C x 7 days

2. At the end of the required incubation period, perform a total colony count per media.
3. 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
4. Identify organism only if requested.

III. 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"

TML/MSH Microbiology Department Policy & Procedure Manual	**Policy # MI\STER\12\v01**	Page 1 of 1
Section: **Sterility Testing Manual**	Subject Title: **Hemodialysis Water Sterility Testing**	
Issued by: **LABORATORY MANAGER**	Original Date: July 2, 2003	
Approved by: Laboratory Director	Revision Date:	

HEMODIALYSIS WATER STERILITY

I. Introduction

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

II. Specimen Collection and Transport

1. Collect a minimum of 10 mL of water aseptically into a sterile container large enough to hold the entire sample with ample of air space to allow for mixing. Avoid any splashing.
2. Deliver the sample to the Microbiology Lab. immediately or refrigerate the sample at 4 - 6^{o}C and deliver it to the Microbiology Lab within 24 hours of collection.

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 BHI Agar plate and spread the inoculum over the entire agar surface.
5. Incubate the BHI plate at 35^{o}C x 48 hrs
6. Remove the plate from the incubator after 48 hours of incubation.
7. 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”