TABLE OF CONTENTS

INTRODUCTION

The validity and accuracy of laboratory test results are dependent upon the quality to the specimen and its careful collection.

Using the collection guidelines, ensure proper sample collection by:

- proper collection site / collection technique
- Correct media/container used
- In-date (not expired) transport media used
- Tightly sealed container (no external spillage)

See Specimen Rejection Criteria QPCMI06001 for sample rejection criteria

Samples should NOT be used for any other purpose than clinical requested tests.

Specimen Labelling

Criteria exist for proper primary sample labelling to ensure optimal specimen processing within the laboratory.

1. All primary specimen containers must be labelled with at least two patient-specific identifiers.
   This is inclusive of patient name, date of birth, hospital number, unique other number, accession number.
2. For specimens were site of origin is critical to analysis, it must be clearly identified on the container and/or requisition and be linkable together.
3. Labels should be placed lengthwise and not wrapped around the containers to permit barcodes to be easily scanned.

See Specimen Rejection Criteria QPCMI06001 for sample rejection criteria for mislabelled / unlabelled samples.

BLOOD CULTURE

A set of blood cultures consists of two Blood culture bottles, one for aerobic and the other for anaerobic culture taken from a single venipuncture site. For infants, a single aerobic pediatric bottle is required.
NOTE: Timing of the collection of blood culture in relation to maximal temperature (fever spike) has not been found to be important in the yield of positive blood culture during a septic episode. Blood culture collection prior to administration of antimicrobials is essential for optimal organism recovery.

Blood collection and transport:

(1) Draw each set of blood cultures (i.e. aerobic and anaerobic bottles) from a separate venipuncture site.

(2) Clean over the proposed site (an area with a diameter of about 5cm) using 2% chlorhexidine with 70% alcohol swabs for 30 sec and allow the site to air dry (usually about 10 sec). Be sure not to palpate or touch the cleaned proposed site with non-sterile gloves after cleaning it.

(3) Remove the protective plastic cap from each bottle and disinfect the rubber septum with 70% alcohol for 10 sec and allow it to air dry before proceeding.

(4) Collect blood using a syringe to assure that the appropriate volume is collected.

(5) For adults, collect 20 mL of blood for each blood culture draw. Expel all air from the syringe and aseptically inject 10 mL into the aerobic bottle and 10 mL in the anaerobic bottle. Do not overfill the bottles.

For pediatric patients, collect the following volume per each blood culture draw:

- <1kg (<2 lbs): 1-2 mL of blood
- 1-2 kg (2-4 lbs): 2-3 mL of blood
- 2-13 kg (5-30 lbs): 3-4 mL of blood
- A13-36 kg (30-80 lbs): 5-8 mL of blood

- >36 kg (>80 lbs): 16-20 mL of blood

(6) For volumes 1-4 mL, expel all air from the syringe and aseptically inject the volume into a Pediatric bottle.

For volumes 5-8 mL, expel all air from the syringe and aseptically inject the volume split evenly between 2 Pediatric bottles.

For volumes 16-20 ml, expel all air from the syringe and aseptically inject 8-10 mL into the the aerobic bottle and 8-10 mL in anaerobic bottle. Do not overfill the bottles.

(7) Transport specimens to the laboratory as soon as possible and incubate at 35°C until loaded onto the BacT/Alert machine. **NB: If a delay in transport or processing is anticipated, DO NOT refrigerate the bottles – leave them at room temperature.**

(8) Special Requests:

i. **Subacute Bacterial Endocarditis/Infective Endocarditis (SBE/IE) and Pyrexia of Unknown Origin/Fever of Unknown Origin (PUO / FUO)**
Collect blood in BacT/Alert blood culture bottles.

ii. **Bone Bank Blood**
Collect blood in BacT/Alert blood culture bottles.

iii. **Fungus and Yeast**
Collect blood in BacT/Alert blood culture bottles.

iv. **Dimorphic Fungi** (e.g. Histoplasma, Blastomyces and Cryptococcus)
Collect blood into the Isolator 10 microbial tubes and **NOT** in BacT/Alert bottles. Process specimen as outlined in ISOLATER 10 BLOOD CULTURE SYSTEM FOR DIMORPHIC FUNGI Procedure. If a delay in processing is anticipated, hold tubes at room temperature for up to 24 hours and process as soon as possible.

If BacT/Alert bottles are received with a request for dimorphic fungi or cryptococcus, **notify the ward/physician** that they must use the Isolator 10 collection tubes. Process the BacT/Alert bottles as per routine blood cultures.

v. **Brucella**
Collect blood in BacT/Alert blood culture bottles. Label bottle as “Brucella”.

vi. **Sterile body fluids**
Collect sterile body fluid in a clean, sterile container. If collection of sterile body fluids in BacT/Alert bottles is desired, collect the specimen in BacT/Alert bottles. In the LIS, add "fluid in BC Bottle" test to the order/entry screen (see Handling of Sterile Body Fluids in Blood Culture Bottles).

vii. **Bone marrow (Sterility testing)**
Collect Bone marrow a clean, sterile container. If collection of sterile body fluids in BacT/Alert bottles is desired, collect the specimen in BacT/Alert bottles. In the LIS, add "fluid in BC Bottle" test to the order/entry screen (see Handling of Sterile Body Fluids in Blood Culture Bottles). For Bone marrow received in a sterile container **DO NOT** inoculate it into BacT/Alert bottles.

Isolator 10 – Blood Culture System for Dimorphic Fungi

With aseptic technique, collect 10 mL of blood into a clean, sterile syringe. Transfer the blood into an Isolator 10 microbial tube. Transport to the laboratory immediately for processing. If a delay in transport or processing is anticipated, the tubes can be held for 24 hours at room temperature.

ENTERIC SPECIMENS

Faeces/Rectal Swabs

Collect a single stool specimen in Copan Fecal Swab (Cary-Blair transport medium may be used if Copan Fecal Swab is not available) and transport it to the laboratory for culture and sensitivity. When faeces cannot be obtained, a rectal swab is acceptable **except** for *Clostridium difficile* toxin assay. Collect the specimen with a sterile swab inserted approximately one inch beyond the anal sphincter and place it in Amies transport medium.

Collect Stool specimens for *C. difficile* toxin assay in a clean, sterile container.

If Campylobacter other than *C. jejuni/coli* is required, collect the specimen in a clean, sterile container and the specimen will be forwarded to the Provincial Health Laboratory for testing.

Collect rectal swab for GC with a sterile swab place it in Amies transport medium.

Collect specimens for *Chlamydia* detection or virus isolation in viral transport media.

Collect specimen for ova and parasites (O&P) in SAF transport medium. Collect directly into an appropriate preservative. Contamination with water or urine must be avoided.

Rectal/Large Bowel (Colon) Biopsies

Collect specimen via a colonoscope or sigmoidoscope and transport in a clean sterile container with a small amount of sterile saline or sterile water or viral transport media. If a delay in transport or processing is anticipated, keep the specimen at 4^{o}C.

Duodenal or Small Bowel Aspirate / Swab / Biopsy

Aspirates should be collected and transported in a syringe (needle removed) or a clean, sterile container. Biopsy specimens should be collected and transported in a clean, sterile container. Duodenal swabs should be transported in Eswab Amies transport medium. Transport specimen, needle removed if syringe is used, to the Microbiology Laboratory ASAP. If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C.

GENITAL SPECIMENS

Cervical Swabs

Collect specimen for GC from the endocervical canal using a clean, sterile swab and transport the swab in Amies transport medium. If there is a delay in transport, store the swab at room temperature.
Collect specimen for GC/Chlamydia in a Cobas PCR swab Container.

Group B *Streptococcus* Screen

A swab obtained from the combined introital (vaginal) and anorectal areas should be collected in Amies transport medium. Cervical and vaginal swabs are not recommended for this type of culture but will be processed if received in the laboratory.

Vaginal Swab for Vaginitis/Vaginosis Screen

Collect swabs from the posterior vaginal vault or vaginal orifice and transport the swab in Amies transport medium. Specimen should be transported to the laboratory as soon as possible. The yield of wet mount for *Trichomonas vaginalis* is significantly diminished if slides are not examined within 15 minutes of collection.

Vaginal Swab for Culture

Collect swabs from the posterior vaginal vault or vaginal orifice and transport the swab in Amies transport medium. Specimen should be transported to the laboratory as soon as possible.

Vaginal swabs are not recommended for GC culture. However, if specifically requested, GC cultures will be set up.

Vaginal Swab for Chlamydia / GC

Collect specimen for GC/Chlamydia PCR in a Cobas PCR swab Container.

Vaginal swab for cases of sexual abuse/ toxic shock / children

Collect swabs from the posterior vaginal vault or vaginal orifice and transport the swab in Amies transport medium.

Urethral Swab

Exudate from the urethra should be collected using a clean, sterile swab and transported in Amies transport medium.
For Chlamydia trachomatis, collect in Viral Transport media.

Penis Swab

Penile swabs should be transported in Amies transport medium.

Seminal Fluid

The periurethral area and hands should be washed and urine should be passed immediately before seminal fluid collection. Using as much of an aseptic technique as possible, seminal fluid should be collected by masturbation directly into a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at room temperature until processing.

Collect specimen for GC/Chlamydia PCR in a Cobas PCR swab Container.

Endometrial Swabs, Biopsies and Curettings, Placenta Swab/Tissue, Products of Conception, Endometrial/Uterine, Cul de sac/Transvaginal, Fallopian Tube, Tubo-Ovarian Swabs or Aspirates

Collect scrapings and small tissue samples, aspirates or swabs aseptically, avoiding lower genital tract contamination, and transport the sample in sterile saline and an anaerobic container.

Genital Ulcer Swab

Refer to Ministry of Health Specimen Collection Guide

Intra-Uterine Device

Collect and transport IUD in a dry, sterile container.

INFECTION CONTROL SCREENING SPECIMENS

Methicillin Resistant *Staphylococcus aureus* (MRSA) Screen

Moisten a sterile swab and rotate inside/over each site to be sampled. Place the swab in Amies transport medium. If a delay in transport or processing is anticipated, keep the swab at 4°C.

Vancomycin Resistant *Enterococcus* (VRE) Screen

Rotate a sterile swab in the rectum or other site of suspected VRE carriage. Place the swab in Amies transport medium for transport. If a delay in transport or processing is anticipated, keep the swab at 4°C.

ESBL and Carbapenemase Screen

Rotate a sterile swab from site suspected of ESBL/Carbepenemase. Place swab in Amies transport medium. If a delay in transport or processing is anticipated, keep the specimens at 4°C.

Resistant Gram Negative Bacilli

Any specimen may be submitted, although rectal swabs and environmental are the most common. Rotate a swab inside/over the suspected site. Swabs should be transported in Amies transport medium. If a delay in transport or processing is anticipated, the swabs should be kept at 4°C.

Klebsiella oxytoca or *Klebsiella pneumonia Screen*

Any specimen may be submitted, although rectal swabs are the most common. Rotate a sterile swab in the rectum or other site. Place the swab in Amies transport medium for transport. If a delay in transport or processing is anticipated, the swabs should be kept at 4°C.

Resistant *Pseudomonas aeruginosa* Screen

Specimens suitable for culture are:

Water samples

Environmental samples
Patient pharmaceutical infusates/injectables
Swabs from patients

Specimens are to be collected by Infection Control Practitioners

Specimen	**Collection**
Water	Fill up a 50 mL Sterile Falcon Centrifuge tube
Environmental Swabs	Swab the area and place the swab into a tube containing 2 mL of BHI broth
Patient pharmaceutical infusates/injectables	Send the original vial
Soaps/creams/thick fluids	Collect the sample with a sterile swab and place the swab into a tube containing 2 mL of BHI broth
Swabs of patients	Swab the desired area and place the swab into Amies transport medium.

Transport specimen at room temperature. If a delay in transport or processing is anticipated, keep the specimens at 4^{o}C.

Group A Streptococcus Screen

Rotate a sterile swab in of suspected GAS site, most commonly from the throat, rectum or wound site. Place the swab in Amies transport medium. If a delay in transport or processing is anticipated, keep the swab at 4^{o}C.

MOLECULAR DIAGNOSTICS SPECIMENS

Refer to the for Specimen collection information.

MYCOLOGY SPECIMENS

Specimens should be collected and transported in a properly labelled, sealed, sterile container. For proper collection and transport of all specimens refer to the appropriate sections of this manual. For cutaneous and skin structure specimens see below.

Cutaneous Specimens:

Specimens of hair, nail and skin scrapings may be placed in the folded black paper found in the Public Health Lab "FUNGUS" mailing container. Alternatively, specimens may also be transported in a dry sterile container or sealed paper envelope.

Process proficiency and any special request: include processing in mycology. Provide labels and media.

Instructions for collecting cutaneous specimens:

The areas to be sampled should be wiped with 70% alcohol to remove surface bacterial contaminants.

Skin: Skin lesions should be sampled from the erythematous, peripheral, actively growing margins of typical "ringworm" infections. Skin scales may be flaked off using a surgical blade.

Nail: Scrape away the superficial portions with the side of a surgical blade before collecting a deeper sample.

Hair: Infected hairs can be plucked with surgical forceps.

If a delay in transport or processing is anticipated, store all specimens except CSF, hair, skin and nails at 4^{o}C. CSF, hair, skin and nail specimens should be kept at room temperature.

RESPIRATORY SPECIMENS

Bronchoalveolar Lavage (BAL), Bronchoscopy Aspirates/Washings – Routine

Collect approximately 15-30 mL of fluid and transport it to the laboratory in a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

CMV Surveillance Bronchoscopy Specimens

Collect approximately 15-30 mL of fluid and transport it to the laboratory in a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Bronchial Brush Specimens

The protected brush-catheter is placed into a screw capped (bijou) bottle containing 1 mL of Ringer's Lactate. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Epiglottal Swabs

Collect epiglottal swab with a clean, sterile swab and swabbing the cartilaginous structure that overhangs the larynx. Submit the swab in Amies transport medium.
If a delay in transport or processing is anticipated, keep the specimen at 4°C.

If viral isolation is required, submit the swab in viral transport medium and forward the specimen to the Virology Section for processing.

Open Lung/Transthoracic Needle/Transbronchial Lung Biopsies/Lung Aspirates

Collect the specimen into a clean, sterile container and transport it to the laboratory immediately. If anaerobic culture is required on a transthoracic needle biopsy, place the specimen in anaerobic transport medium. If a delay in transport or processing is anticipated, add 0.5 mL of sterile saline and keep the specimen at 4°C.

If virology is required, forward a portion of the specimen to Virology for processing.

Forward a portion of all specimens to the Public Health Laboratory (PHL) for Mycobacteria (TB) culture.

Mouth Swabs

Swab lesions in the mouth using a clean, sterile swab and then place the swab in Amies transport medium. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Nasal Swabs

Using a saline moistened swab, vigorously rotate just inside each nostril and transport the specimen in Amies transport medium. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Nasopharyngeal Swabs/Auger Suctions for *Bordetella pertussis*

Collect a posterior nasopharyngeal swab and placed in a clean, sterile container. Routine throat swabs are not acceptable. Collect auger suctions using a specialized syringe and tubing. The tubing should be sent to the lab in a sterile container.

Oral Abscess Swabs

Collect specimen using a clean, sterile swab from mouth or dental abscess and place the specimen in Amies transport medium. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Sinus/Antral Specimens

Collect sinus aspirate and antral lavage into a clean, sterile container. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Sputum including Endotracheal tube and Tracheotomy Specimens

Obtain sputum specimen from a deep cough and expel into a clean, sterile container.

Collect sputum samples from patients with tracheostomies and endotracheal tubes (ETT) using a suction device and a Lukens trap.

Send small volumes of endotracheal tube (ETT) secretions from neonates in the suction tubing.

An ETT tip from an adult is an unacceptable specimen.

If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Throat Swabs

Collect the specimen using a clean, sterile swab and place it in Amies transport medium.
If a delay in transplant or processing is anticipated, keep the specimen at 4°C.

Gastric Aspirates/Biopsies

Collect specimen into a clean sterile container and transported to the laboratory as soon as possible. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Gastric Aspirates/Biopsies for *Helicobacter Pylori*

Portagerm pylori (*Helicobacter pylori* transport medium) collection container must be requested through the Department of Microbiology at minimum 1 month prior to scheduled surgery and stored at 2-8C.

For optimal results, each sample should comprise at least 4 biopsies (two from antrum & two from corpus) .

Collect specimen into Portagerm pylori transport medium for *H. pylori* ensuring sample is deeply plunged into the transport medium immediate after collection.

Transport samples to the laboratory within 4 hours of the biopsy.

SEROLOGY SPECIMENS

Refer to the Serology Test Manual for Specimen collection information.

STERILE BODY FLUID SPECIMENS

Cerebrospinal Fluids

Collect the specimen into a clean, sterile, leak-proof centrifuge tube and transport immediately to the laboratory. Collect several tubes to avoid delay in processing the specimen in the various laboratories.

- If multiple tubes have been collected, avoid using 1st tube for microbiological examination
- Send 1-2 mL of specimen to the Microbiology department for culture.
- If fungal or mycobacterial meningitis is suspected, send an additional 3 to 4 mL to the microbiology laboratory.
- If a delay in transport or processing is anticipated, keep the specimen at **room temperature**.

Other Sterile Fluids – Amniotic, Pleural (Thoracentesis/Empyema), Peritoneal (Ascites), Synovial (Joint), Pericardial, Tympanocentesis, Intraocular, Hydrocele Fluids etc

Collect the specimen by aseptic percutaneous aspiration and transport it in a clean, sterile container and an anaerobic transport container. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Peritoneal Dialysis Effluent

Drain the dialysis solution from the patient's abdomen directly into bags and transport the entire bags to the lab as soon as possible. If a delay in transport or processing is anticipated, keep the bags at **room temperature**.

Predialysis Fluid

Collect 5 mL of predialysis fluid aseptically into a red top Vacutainer tube and transported it to the lab as soon as possible. If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Amniotic Fluids

Collect amniotic fluid by amniocentesis, Caesarean section or by aspiration with a transcervical intrauterine catheter. Transport the fluid in a clean, sterile container and/or anaerobic transport container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Bone Marrow (Aspirates or Biopsies)

A. Bone marrow aspirate:

1. Bone marrow aspirate for C&S, fungal, or mycobacterial culture:

For C&S, collect a minimum of 0.5 mL (preferably 3ml) directly into pediatric blood culture tube and an equal volume into an anaerobic blood culture bottle

For fungal and mycobacterial (and C&S if specimen not collected in PEDC and FNV blood culture bottles), collect a minimum of 0.5 ml (preferably 3ml) in a Sodium Polyanethol (SPS)

tube (light yellow top to be stocked in micro) for each culture requested (preferably larger volume for fungal and mycobacterial cultures)

2. Bone marrow aspirate for NAAT (16S or fungal NAAT) (NB: PHOL will not accept BM aspirates for TB PCR):

Collect a minimum of 0.5 ml in an EDTA tube (purple top) for each NAAT requested

B. Bone marrow core biopsy:

1. Bone marrow core biopsy for C&S, fungal, or mycobacterial culture:

Collect in a sterile container on a sterile saline soaked gauze

2. Bone marrow core biopsy for NAAT (16S, fungal or TB NAAT) (preferred specimen for NAAT compared with BM aspirate):

Collect in a sterile container on a sterile saline soaked gauze

Blood, Platelets, and Other Transfusion Products

Send the suspect contaminated transfusion bag to the lab intact. If a delay in transport or processing is anticipated, keep the bag at 4^{o}C.

STERILITY SPECIMENS:

Microbial Enumeration (Bioburden) Samples

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

For products where the total number of entities in a batch is less than 200, the samples size may be reduced to two units, or one unit if the size is less than 10.
Samples should be chosen at random from the bulk material or available containers. To obtain the required quantity, mix the contents of a sufficient number of containers to provide the sample.

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

A negative control must be performed to verify testing conditions. Inoculate an equal portion of chosen sterile diluent in place of the test preparation.

Each TSB will compromise one test and shall be labelled with a unique identifier.

Send samples at room temperature without delay for testing.

BIOLOGICAL SPECIMENS:

Bone Bank Specimens

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

Bone Bank Specimens - Fresh Osteochandral Allograft

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.

Cardiovascular Lab Specimens (Dog)

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

Medicinal Leech Testing

Leech specimen should be submitted in a sterile container with minimal leech water
Leech storage fluid should be submitted in a sterile container
Leech vendor fluid should be submitted in a sterile container
Leech container should be swabbed and submitted in Aries transport media

Tissue Cultures Specimens for Injection

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

NON-BIOLOGICAL SPECIMENS:

Air Sampling by Air Flow Sampling Apparatus

Air sampling specimens are collected on various media depending on the purpose of the area to be measured and the type of organisms to be counted. Culture media will be subjected to a specified volume of airflow and be submitted to the microbiology lab for incubation and colony count.

Type of organism	Media
Bacteria	Blood Agar
Fungi	Inhibitory Mold Agar
Bacteria Fungi	Trypticase Casein Agar
Fungi	Rose Bengal Agar

Attest

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

Chemspore / Sterikon

Submit the ampoule intact until activated in the lab.

Contact Lens & Solution

Collect specimen in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C. If Acanthamoeba is requested, collect specimen into Acanthamoeba collection saline (Pages Saline) forward specimen to PHL for processing. If there is a delay in transport, store the specimen at room temperature for no longer than 12 hours.

Distilled/De-Ionized Water Sterility

Open the water tap fully and allow the water to run for a minimum of 1 minute before sampling. 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.

Endoscope Surveillance Swabs

10mL of sterile saline if flushed through the instrument channel port and collected into a 15mL conical tube.

The inlet ports of the scope will be brushed and swabbed with a flocked swab. The swab will be broken off into the conical tube with the flushed saline.

A second swab will be used to brush the elevator channel mechanism in the duodenoscopes and will be placed in the conical tube.

Transport specimens at room temperature to the microbiology laboratory. Store at 4^{o}C for delayed processing.

Environmental Monitoring

Air Sampling by Settle plate

1. Place inverted, culture media plates (Tryptone Soya Agar for bacteria, Inhibitory Mold Agar for fungi) around the sample processing area
2. Remove lids from the media plates
3. Leave the media until the clinical sample processing is complete or replace with new media every 4 hours or designated sampling period.
4. Close the lid of the settle plates and send to microbiology for incubation and testing.

Contact plate sampling

1. Remove lid of contact plate (Tryptone Soya Agar e.g. RODAC plate) and touch the contact plate to the surface area to be sampled for testing
2. Replace lid when done and send sample to microbiology for incubation and testing
3. When sampling is complete, clean the sampled area prior to use

Glove prints contact plate

1. Periodically, product processing staff glove prints are sampled for colony counts. Frequency of sampling is determined by the individual lab submitting the samples.
2. Staff gloved fingers are touched to culture media (Tryptone Soya Agar) at various stages of the processing as determined necessary by the submitting lab.
3. Each hand (fingers and thumb) should be gently touched and rolled onto a different agar prior to any glove disinfection. A slight impression should be left on the agar.
4. Remove and change glove when complete and send sample plate(s) to microbiology

Media Fill

1. The simulation chosen must be representative of real compounding conditions and represent the most complex preparations according to the risk level of preparations made.
2. Sterile media (eg. Tryptone soya broth) is used for low or medium risk procedures. For high risk procedure, medium must be non-sterile and include sterilization by filtration.
3. Aliquots of sterile TSB are transferred to clear vials. Sterile adhesive seals shall be affixed aseptically to the rubber closures on the three filled vials and submitted to the laboratory promptly.

Hemodialysis Water Sterility

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.

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.

Hemodialysis Ultrapure Dialysis Fluid Sterility

For instruments with sampling ports, disinfect port with alcohol and allow to air dry. A sterile syringe should be used to aspirate at least 10ml of dialysis fluid. Discard filled syringe and collect a fresh sample using a new sterile syringe.
For sample ports consisting of a simple septum penetrated with a needle, the use of second syringe is not necessary.
Alternatively, samples can be collected immediately before the dialyser by disconnecting the inlet connector and aseptically collecting a “free/clean” catch sample after allowing dialysis fluid to run for at least 60 s unless manufacturer instructions state otherwise.

Deliver to the Microbiology laboratory immediately or store and transport at <10 ^{o}C without freezing beyond 4 hours of collection. Sample storage beyond 24 hours should be avoided.

Miscellaneous Non-biological Samples

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

Product Sterility – Compendial method

1. Collect and prepare specimen as per pharmacy departmental policies and procedures and established guidelines for product sterility testing.

   Sampling of lots
   Samples for sterility testing are submitted by pharmacy with the minimum number of articles to be tested in relation to the number of articles in the batch and the minimum quantity of product to be tested from each container as per USP <71> . See Table 1 and Table 2.

Table 1: Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Number of Items in the Batch	**Minimum Number of Items to be Tested for Each Medium (unless otherwise justified or authorized)**
Parenteral preparations	
Not more than 100 containers	10% or 4 containers, whichever is the greater
More than 100 but no more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less

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

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

3. The facility is responsible for ensuring submission of a negative control using a chosen diluent in place of the test preparation to verify collection and testing conditions. Submit this sample to the lab following the same product sterility instructions.

4. For each product requiring sterility testing, affix the same product information or negative control label onto both the Thioglycollate broth tube and Tryptone Soya broth tube

5. Order a sterility test and document specimen collection in the hospital electronic information system (EPR) for each product or complete a provided requisition.

6. Using the specimen number generated by the hospital information system (EPR), label the pharmaceutical product, Thioglycollate and Tryptone Soya tubes. For orders provided through requisitions, label tubes with a minimum of two unique product identifiers.

7. Using a 3ml syringe and aseptic technique remove 2ml of the pharmaceutical product from original specimen bottle/vial

8. Inoculate 1 mL of the aspirated specimen into the Thioglycollate tube and 1mL into the Tryptone Soya tube

9. Place inoculated Thioglycollate broth and Tryptone Soya broth tubes back into original shipping container or foam rack and send to Microbiology for incubation and culture.

10. Store remaining of the pharmaceutical product in 4^{o}C refrigerator for 1 month in pharmacy until sterility testing result is finalized and reviewed

11. For repeat sterility testing, retrieve the remaining stored pharmaceutical product and follow steps #2 to #10 for preparation of the repeat sample

    Note to use the same lot number of the Thioglycollate and Tryptone Soya broths as the original submitted broths when sending the repeat sample. Send also a set of uninoculated Thioglycollate and Tryptone Soya broths of same lot numbers. Microbiology will provide the lot numbers when requesting for the repeat sample for sterility testing

12. For step #3, ordering of the repeat sterility test in EPR, indicate in the new order, the repeated product identifier and the original EPR specimen number as :

    "Repeat for <u>product xxxx</u> , <u>EPR #xxxxxx</u> "

Product Sterility – Rapid BacT/Alert Method

1. Collect and prepare specimen as per pharmacy departmental policies and procedures and established guidelines for product sterility testing.

   Sampling of lots
   Samples for sterility testing are submitted by pharmacy with the minimum number of articles to be tested in relation to the number of articles in the batch and the minimum quantity of product to be tested from each container as per USP <71> . See Table 1 and Table 2.

Table 1: Minimum Number of Articles to be Tested in Relation to the Number of Articles in the Batch

Number of Items in the Batch	**Minimum Number of Items to be Tested for Each Medium (unless otherwise justified or authorized)**
Parenteral preparations	
Not more than 100 containers	10% or 4 containers, whichever is the greater
More than 100 but no more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less

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

2. The facility is responsible for ensuring submission of a negative control using a chosen sterile diluent in place of the test preparation to verify collection and testing conditions. Submit this sample to the lab following the same product sterility instructions.

3. Order a rapid sterility test and document specimen collection in the hospital electronic information system for each product or complete a provided requisition.

4. Using the specimen number generated by the hospital information system , label the pharmaceutical product *i*FA and *i*FN bottle. For orders provided through requisitions, label tubes with a minimum of two unique product identifiers.

5. Samples must be collected aseptically and maintained under sterile conditions prior to testing.

6. Visually inspect bottle prior to inoculation. Do NOT use bottles with:
    - Evidence of damage, leakage or deterioration
    - Evidence of broth turbidity, excess gas pressure (observe for bulging septum), a yellow indicator, and/or evidence of growth.

7. Ensure bottles are at room temperature. Disinfect septum with an alcohol pad or equivalent

8. DO NOT fill above bottle's maximum sample volume of 10mL

9. Inoculate bottle by direct inoculation method recommended for liquids
    - A liquid sample may be inoculated into the bottles through the rubber septum via a needle and syringe.
    - Do NOT vent bottles
    - Avoid multiple inoculations into bottle
    - Recommend a ***23-gauge needle or smaller*** to prevent disruption to internal bottle environment
    - Clean septum with alcohol or equivalent prior to inoculation

10. Submit inoculated samples (and requisition) to Microbiology for incubation and culture promptly.

11. Store remaining of the pharmaceutical product in 4^{o}C refrigerator for 1 month (or as required) in facility until sterility testing result is finalized and reviewed or in case repeat testing is required.

Note to use the same lot number of the bottles as the original submitted bottles when sending the repeat sample. Send also a set of uninoculated iFA & iFN bottles of same lot numbers. Microbiology will provide the lot numbers when requesting for the repeat sample for sterility testing

Spore Strip

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

URINE SPECIMENS

Urine is normally a sterile body fluid. However, unless it is collected properly, it may become contaminated with normal flora from the urethra, vagina, prostate or perineum.

Collect urine specimen into a clean, sterile container, then transfer it to a BD grey top urine vacutainer tube. A sterile container is also acceptable. Transport the specimen to the Microbiology Laboratory ASAP if collected in a sterile container. Processing of specimens within 2 hours of collection is recommended. If a delay in transport or processing is anticipated, place the specimen in the refrigerator (4^oC to 8^oC) until processed.

Voided Urines:

Midstream urine (MSU)

First, carefully clean the urethral meatus. Then void and discard the first 10 - 20 mL of urine in order to clear the urethra. Collect the subsequent urine into a clean, sterile container.

Neonatal bagged urine

Place a clean collection bag over the external genitalia. Transfer the urine from the bag into a clean, sterile container.

Indwelling catheter (Foley catheter) urine

Puncture the catheter tubing aseptically and transfer the urine into a clean, sterile container.

Ileal conduit urine

Clean the stomal opening with alcohol. Insert a sterile catheter to collect the urine and transfer the urine into a clean, sterile container.

In and Out Catheter / Catheter Insertion Urine

Collect urine into a clean, sterile container immediately following the initial insertion of an indwelling catheter into the bladder.

Aseptically Collected Urines

Nephrostomy urine

Urine draining from a nephrostomy tube placed in the renal pelvis is collected into a clean, sterile container.

Bladder / Cystoscopy / In and out catheter urine

Collect urine into a clean, sterile container following temporary insertion of a sterile catheter or cystoscope into the bladder.

Suprapubic urine aspirate

Aspirate urine through the bladder using a sterile needle and syringe. Transfer the urine into a clean, sterile container.

Segmented Urines

Collect these specimens for the diagnosis of chronic bacterial prostatitis. Collect three urines plus **prostatic secretions** and designate them as follows:

VB_1 = first voided urine representing the urethra
VB_2 = midstream urine representing the bladder
VB_3 = first voided urine after prostatic massage representing the prostrate
EPS = expressed prostatic secretions

Special Requests:

Eosinophil Stain –

Collect first voided urine into a clean, sterile container and transport the specimen to the laboratory ASAP.

Bacterial Latex Agglutination –

Bacterial antigen test is not done due to poor sensitivity and specificity.

Anaerobes –

Collect urine into clean sterile container and transport the specimen immediately to the laboratory. Appropriate specimen is bladder suprapubic aspirate.

Chlamydia Detection –

Collect first voided urine into a Cobas Urine PCR media tube or sterile container and transport the specimen to the microbiology laboratory immediately.

Legionella Antigen Detection –

Collect urine into clean sterile container and transport the specimen to the laboratory ASAP. Testing will be performed by the Provincial Health Laboratory.

Leptospira Detection –

Collect urine into clean sterile container and transport the specimen to the laboratory ASAP. Notify microbiologist. Testing will be performed by the Provincial Health Laboratory.

Cryptococcus/Systemic Fungi –

Collect urine into clean sterile container and transport the specimen to the laboratory ASAP.

TB Culture –

Collect first morning voided urine on three consecutive days into clean, sterile containers. Testing will be performed by the Provincial Health Laboratory.

Viral Culture –

Collect urine into a clean, sterile container and transport the specimen to the Virology laboratory immediately.

Parasitology – *Schistosomiasis* –

Collect mid-day urine into a clean, sterile container and transport the specimen to the Parasitology laboratory ASAP.

WOUND/TISSUE/ASPIRATE SPECIMENS

SWABS AND DRAINAGE SPECIMENS:

Intraoperative/Interventionial Swabs

Intraoperative and interventional swabs should be an ESwab (Liquid Amies Elution swab). If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C

Wound/Abscess Swabs and Drainage

Specimens should be collected using a sterile ESwab (Liquid Amies Elution swab). Drainage material should be collected into a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C.

Bite Wound Swabs

Specimens should be collected using a clean, an ESwab (Liquid Amies Elution swab). If a delay in transport or processing is anticipated, the aerobic swab should be kept at 4^{o}C and the anaerobic swab at room temperature.

Intravenous & Central Line Catheter Exit Site Swabs

Specimens should be collected using a clean, an ESwab (Liquid Amies Elution swab). If a delay in transport or processing is anticipated, keep the specimen at 4^{o}C

ABSCESS SPECIMENS (Not Swabs):

Intraoperative/Interventionial Abscess (Pus/Cyst Fluid or Aspirate)

Intraoperative and interventional aspirates should be sent in sterile container or an ESwab (Liquid Amies Elution swab). If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C.

Pus & Abscess Material (other than Intraoperative/Interventional, Rectal or Bartholin

Pus from an abscess should be sent in a clean, an ESwab (Liquid Amies Elution swab). If a delay in transport or processing is anticipated, keep the specimen at 4°C.

Rectal Abscess

Rectal abscess swabs should be an ESwab (Liquid Amies Elution swab). Pus from a rectal abscess should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Bartholin's Abscess swab/Aspirate

Specimens for culture are collected should be an ESwab (Liquid Amies Elution swab). For detection of CT/GC, refer to the.

TISSUES, PROSTHETIC DEVICES, AND AUTOPSY SPECIMENS:

Tissues/Biopsies (other than skin or transplant tissues)

Tissue should be collected in a clean, sterile container with a small amount of sterile saline. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Skin Biopsies

Skin biopsy specimens should be placed in a sterile container with sterile saline. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Transplant - Bone Graft & Cadaver Fascia/Tissue/ Swab Specimens/Donor Amniotic Fluid/Membrane; Donor Corneal Ring Material

Swabs from the donor bones or fascia should be collected using a clean, sterile swab and sent in Amies transport medium. If anaerobic culture is requested, an anaerobic swab sent in anaerobic transport medium should be collected. Bone or fascia tissue should be sent in a clean, sterile

container. If a delay in transport or processing is anticipated, the aerobic specimen should be kept at 4°C.

Prosthetic Devices (e.g. Pacemaker Wire, Dacron Graft, Prosthetic Valve)

These specimens should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Autopsy Specimens

This specimen should be received in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

CATHETER SPECIMENS:

Intravascular Catheter Tips

These specimens should be sent in a clean, sterile container.
If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Peritoneal Dialysis Catheter/Canula

These specimens should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

BILE SPECIMENS:

Bile and Bile Stents

Bile may be aspirated with a syringe during surgery or collected in a sterile container from a post-op drain. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

MISCELLANEOUS FLUID SPECIMENS:

Breast Milk

Breast milk should be sent in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Total Parental Nutrition (TPN)

A TPN set disconnected from patients with fever consists of a TPN bag, tubing and a lipid bottle. Distinguish these from Pharmacy TPN samples for sterility testing which are sent in small vials. Inform Infection Control Nurse when TPN set is received.

EAR SPECIMENS

Ear Swab

The ear swab should be collected using a clean, sterile swab and sent in Amies transport medium. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Tympanocentesis Fluid

Tympanocentesis fluid is obtained for the diagnosis of otitis media. These specimens are handled as sterile fluids. (Refer to Sterile Fluids Section)

EYE SPECIMENS

Eye / Conjunctival / Lid Swabs

It is preferable that both eyes be swabbed, even if the infection is unilateral. Swabs should be collected prior to the instillation of topical anaesthetics or antibiotics, and sent in Amies

transport medium. Viral isolation requires special transport media. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

Occasionally, specimens collected by an ophthalmologist will be inoculated directly onto culture plates at the bedside. The ophthalmologist will inoculate the plates in a short spiral line. If lid swabs are also collected, these will be inoculated onto the same culture plates next to the conjunctival inoculation. Lid swabs will be inoculated in the shape of an "L" or "R" indicating left or right, respectively. These plates should be kept in the incubator (35°C) until processed.

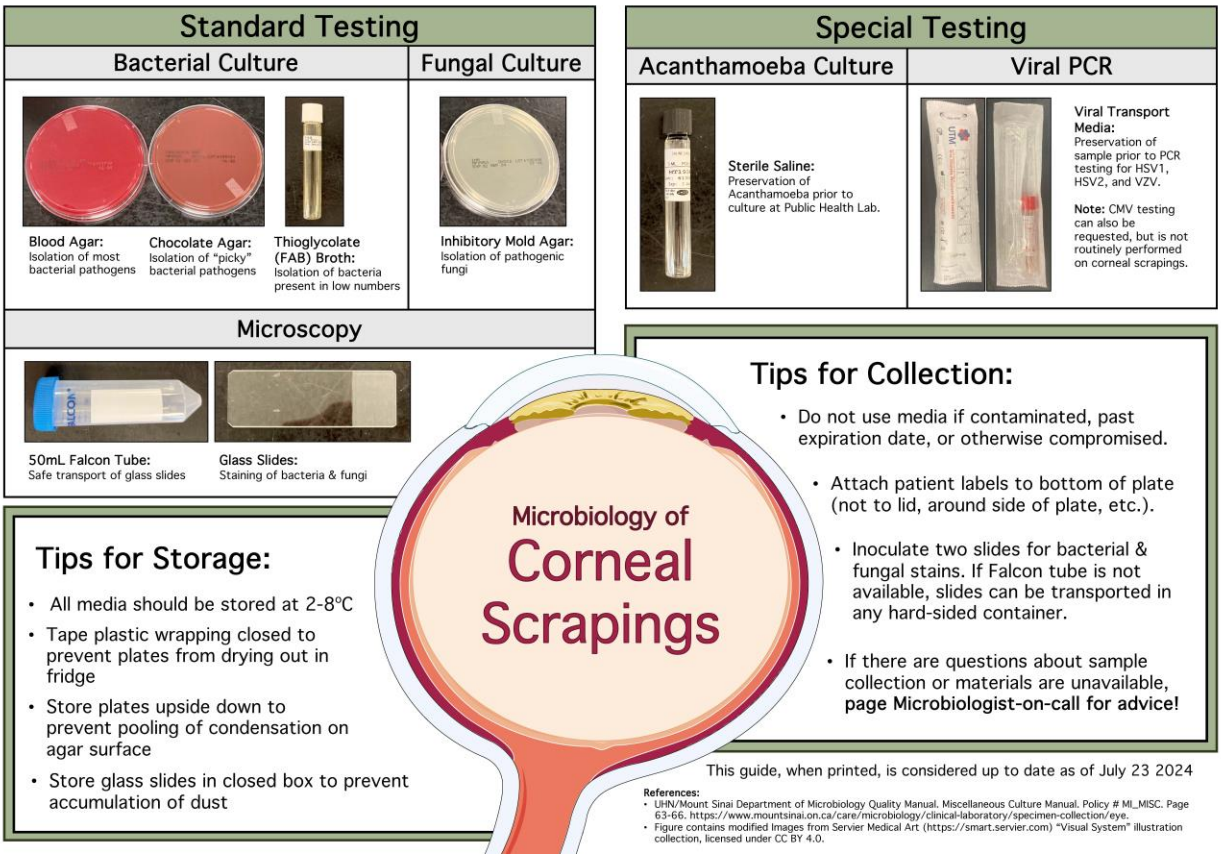
Eye / Corneal Scrapings

The media/supplies needed are listed in the table below:

Testing	**Media/Supplies**
Bacterial Culture	Blood Agar (BA) Chocolate Agar (CHOC) Fastidious Anaerobic Broth (FAB) (aka THIO)
Fungal Culture	Inhibitory Mold Agar (IMA)
Acanthamoeba Culture	Sterile Saline
Viral PCR	Viral Transport Media
Microscopy	50 mL Falcon Tube or other hard-sided container Glass Slides

The media are to be supplied to the physician for each eye: BA, CHOC, IMA and THIO. The physician will inoculate the plates in rows of "C" - shaped marks, with each row representing a separate sample; the inoculated plates should be sent to the microbiology laboratory without delay. Once received in microbiology, if a delay in processing is anticipated, inoculated plates should be kept in the incubator (35°C) in Specimen Management area.
Virus and chlamydia detection require special viral transport media (see Molecular Manual), and should be kept at 4°C if delay in transport or processing is anticipated.
If acanthamoeba is requested, collect specimen in saline and forward specimen to PHL for processing. If there is a delay in transport, store inoculated saline bottle(s) at room temperature.

Follow the chart below for the media/supplies used and the tips for storage and transport.



Intraocular Aspirates

Aspirates of intraocular fluids are submitted for the diagnosis of uveitis and endophthalmitis. These specimens are handled as sterile fluids.
(Refer to Sterile Fluids Section)

Lacrimal (Tear Duct) Stone / Secretions

Specimens are to be collected and transported in a clean, sterile container. If a delay in transport or processing is anticipated, the specimen should be kept at 4°C.

FACIAL SPECIMENS

Facial Swabs

These specimens should be transported in either an anaerobic transport container or Amies transport medium. If a delay in transport or processing is anticipated, the specimen should be kept at 4^{o}C.

Record of Edited Revisions

Manual Section Name: Pre-analytical Procedure - Specimen Collection

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	May 4, 2005	Dr. T. Mazzulli
Annual Review	July 23, 2006	Dr. T. Mazzulli
Annual Review	August 13, 2007	Dr. T. Mazzulli
Blood Culture Collection - revised	June 15, 2008	Dr. T. Mazzulli
Annual Review	June 15, 2008	Dr. T. Mazzulli
Annual Review	June 16, 2009	Dr. T. Mazzulli
Annual Review	May 31, 2011	Dr. T. Mazzulli
Updated Respiratory Section	November 07, 2011	Dr. T. Mazzulli
Annual Review	May 31, 2012	Dr. T. Mazzulli
Annual Review	May 31, 2013	Dr. T. Mazzulli
Annual Review	May 09, 2014	Dr. T. Mazzulli
Added IC sections: ESBL/CRE, Resistant GN, Kleb oxy/pneumo, GAS, Pseudo screen Added Wounds section Added section for Serology and Molecular Diagnostics with links to the manuals for collection and transport information. Added sterility section	May 26, 2015	Dr. T. Mazzulli
Genital specimens: -Added Vaginal Swab for Chlamydia / GC – collect in Cobas PCR tube -Under cervical added: Collect specimen for GC/Chlamydia in a Cobas PCR swab Container. -Under Seminal Fluid added: Collect specimen for GC/Chlamydia PCR in a Cobas PCR swab Container. -added Vaginal swab for cases of sexual abuse/ toxic shock / children**:**Collect swabs from the posterior vaginal vault or vaginal orifice and transport the swab in Amies transport medium.	January 13, 2016	Dr. T. Mazzulli
Annual Review Added link to specimen labelling manual Added introduction section	May 26, 2016	Dr. T. Mazzulli
Added “Endoscope surveillance swab” to	October 28, 2016	Dr. T. Mazzulli

Page Number / Item	Date of Revision	Signature of Approval
Sterility/Nonbiological section		
Annual Review	May 14, 2017	Dr. T. Mazzulli
Medicinal Leech Testing added to biological sterility specimens. Media fill environmental collection instructions added. Merged Radio pharmacy and Pharmacy sterility to follow Radio pharmacy procedure. Section name remains as Pharmacy sterility.	May 14, 2018	Dr. T. Mazzulli
Annual Review Addition to specimen labeling section within Introduction	June 12, 2018	Dr. T. Mazzulli
Updated collection of environmental sterility testing. Include TSA instead of BA. Included procedures for all risk level facilities.	September 4, 2018	Dr. T. Mazzulli
Expanded introduction.	September 13, 2018	Dr. T. Mazzulli
Addition of USP compounding facility collection guidelines	September 26, 2018	Dr. T. Mazzulli
Addition of Gastric biopsy/aspirate for *H.pylori*	November 28th, 2018	Dr. T. Mazzulli
Annual Review Addition of step #3 to Pharmacy Sterilty: "The facility is responsible for ensuring submission of a negative control using a chosen diluent in place of the test preparation to verify collection and testing conditions. Submit this sample to the lab following the same product sterility instructions."	November 27th, 2019	Dr. T. Mazzulli
Addition of rapid sterility testing collection instructions	February 18, 2020	Dr. T. Mazzulli
Addition of Microbial Enumeration Testing	April 8, 2020	Dr. T. Mazzulli
Annual Review	December 29, 2020	Dr. T. Mazzulli

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

Page Number / Item	Date of Revision	Edited by:
Added note in intro regarding "In-date (not expired) transport media used"	Jan 12, 2021	Dorna Zareianjahromi
Added note CSF, avoid using 1st tube for micro	Jan 19, 2021	Dorna Zareianjahromi
Added O&P collection, collected directly into an appropriate preservative. Contamination with water or urine must be avoided.	Feb 05, 2021	Dorna Zareianjahromi
Minor formatting change	April 10, 2021	Jessica Bourke

Page Number / Item	Date of Revision	Edited by:
Updated blood culture collection timing info	April 10, 2021	Wayne Chiu
Updated enteric transport to Copan Fecal Swab	Sep 22, 2021	Wayne Chiu
Added subsection with additional info for bone marrow collection	June 15, 2022	Wayne Chiu
Changed "Recommend a 27-gauge needle....." to "Recommend a 23-gauge needle or smaller ……" in ***Product Sterility – Rapid BacT/Alert Method*** section (page 27) as per bioMerieux BACTALERT change notification	September 19, 2022	Oliver Li
Addition of Section Ultrapure Hemodialysis fluid	April 17, 2023	Jessica Bourke
Updated instructions for "Eye / Corneal Scrapings" and included the infograph for clarity	September 12, 2024	Oliver Li
Minor formatting changes	September 16, 2024	Oliver Li