

MICROBIOLOGY COLLECTION PROCEDURES

Document Number: MIC-103.003

BODY FLUID COLLECTION (abdominal, amniotic, ascites, bile, joint, paracentesis, pericardial, peritoneal, pleural, synovial and thoracentesis)

Always submit as much fluid as possible, up to 20 mL of the specimen for microbiologic analysis.

Unacceptable: a swab that has been dipped in the fluid.

- Collect the fluid using a sterile needle and syringe. Expel air from the syringe immediately after collection.
- If the specimen will be received at NPL within 30 minutes of collection: remove the needle and replace with a sterile cap. Transport the syringe. If there will be a delay in transport, place fluid into anaerobe transport medium.
- 10 mL of fluid may be added to an aerobic Bactec blood culture bottle (recommended for peritoneal dialysate effluent and synovial fluid).
- Transport at room temperature.

CEREBROSPINAL FLUID (CSF) COLLECTION

The provider will have collected an adequate amount of fluid (2-10 ml of CSF is sufficient for bacterial, fungal and acid-fast cultures) using established procedures.

- Place the collected fluid into three sterile leak-proof tubes.
- Cap the tubes tightly.
- Submit the second tube for culture to reduce the possibility of contamination due to skin flora.
 1. For bacterial culture, do not refrigerate, transport immediately at room temperature. If there will be a delay in transport, incubate at 37°C or leave the fluid at room temperature.

Note: If the specimen is collected through a ventricular shunt, it is important to label these specimens as "ventricular shunt fluid" and not "CSF".

EYE/EAR COLLECTION

Ear

A swab is not recommended for collecting specimens to diagnose otitis media infections. The specimen of choice is an aspirate from behind the tympanum (eardrum). A small swab may be used only when the eardrum has ruptured and fluid is collected.

- Cleanse the external ear canal with antiseptic solution.
- Collect using established surgical procedures.
- The provider surgically incises the eardrum and collects as much fluid as possible into a syringe or the material may be allowed to collect on a sterile culture swab.
- For fluid collection: If specimen will arrive at NPL within 30 minutes: remove the needle and cap the syringe. Transport the syringe to the laboratory. If there will be a delay in transport, transfer the fluid collected into anaerobic transport media.
- If using a culture swab: place into anaerobic transport media immediately.
- Transport at room temperature or refrigerated.

Note: If an swab of external ear is collected, place in Aimes or Stuarts transport media.

Eye

If inoculating the sample immediately at the time of collection, please call the laboratory prior to collection to obtain appropriate media.

- Purulent conjunctivitis:
 1. Cleanse the skin around the eye with a mild antiseptic.

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2. Collect purulent material with a sterile culture swab.
 3. Place the culture swab into Aimes or Stuarts transport media.
 4. Transport at room temperature or refrigerated.
- Corneal infections:
 1. Swab the conjunctiva as described above.
 2. The provider will have collected multiple corneal scrapings and may inoculate directly onto agar media. (For bacterial culture: inoculate chocolate and blood agar. For fungal culture: inoculate Inhibitory mold and Potato flake agar.)
 3. Transport at room temperature or refrigerated.
 - Intraocular fluid:
 1. The provider will have collected fluid by surgical needle aspiration.
 2. If specimen will arrive at NPL within 30 minutes: remove the needle and cap the syringe. Transport the syringe. If transport is delayed, utilize Aimes or anaerobic transport system.
 3. Transport at room temperature or refrigerated.

GENITAL CULTURE COLLECTION

Urethral- Female

Collect one hour after patient has urinated.

- Remove exudate from urethral orifice.
- Collect discharge material on swab by massaging urethra against pubic symphysis through vagina.
- Place the culture swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Urethra- Male

Patient should not have urinated \leq 1 hour prior to collection.

- Express and discard any exudate.
- Carefully insert flexible, fine-shafted swab 4 cm into urethra.
- Rotate swab 2-3 times to obtain an adequate number of cells.
- Place the culture swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Bartholin

- Disinfect skin with an iodine preparation.
- Aspirate fluid from ducts.
- Transport fluid in securely capped syringe or anaerobe transport media.
- Transport at room temperature or refrigerated.

Vaginal

- Wipe away excessive amount of secretion or discharge.
- Obtain secretions from mucosal membrane of vaginal vault with sterile swab.
- Place the culture swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Cervix

- Look at cervix through speculum without lubricant.
- Remove mucous and/or secretions from cervix with swab and discard the swabs.
- Firmly, yet gently, sample endocervical canal with sterile swab.

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- Place the culture swab into Aimes or Stuarts transport media.
- Transport room temperature or refrigerated.

Vaginal/Rectal Cultures for Group B Strep

- Collect at 35 – 37 weeks of pregnancy.
- Swab vagina, sampling secretions from the lower one third of the vagina.
- Using the same swab, insert into rectum about ≥ 0.5 cm beyond the anal sphincter.
- Place the culture swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Genital Culture (Viral for HSV)

- Clean lesion with sterile saline and remove the lesion's surface with a sterile scalpel blade.
- Allow transudate to accumulate.
- While pressing the base of the lesion, firmly sample exudate with a sterile swab.
- Place the culture swab into viral transport media (UTM).
- Transport at room temperature or refrigerated.

RAPID INFLUENZA COLLECTION

1. Gently pass the flocked swab through the nose and into the posterior nasopharynx. (Must be a nasopharyngeal collection.)
2. Rotate the swab on the nasopharyngeal membrane 5-6 times and allow it to remain in place for 10-15 seconds.
3. Remove the swab and repeat the procedure in the other nare using the same swab.
4. Remove cover from the UTM, place the swab in the liquid and snap the swab shaft off at the score and leave the swab inside the vial. Replace the cap; be sure to tighten securely to prevent leakage.
5. Transport immediately at room temperature.

KOH AND FUNGUS COLLECTION

KOH

Acceptable specimens: Fluids, Hair Clippings, Skin Scrapings, Tissue, mouth, throat or vaginal.
Minimum sample: Collect enough scraping to cover the head of a thumbtack.

Hair

- Scrape the scalp with a blunt scalpel. (Hair stubs, contents of plugged follicles, and skin scales are also acceptable)
- Hair may also be plucked from the scalp with forceps. (Do not submit cut hair.)
- Place in a dry sterile container.
- Transport at room temperature.

Nails

- Cleanse the nail with alcohol.
- Remove the outermost layer by scraping with a scalpel.
- Clippings from any discolored or brittle parts of the nail may be used.
- Deeper scrapings and debris under the edges of the nail are also suitable.
- Place the specimens in a dry sterile container.
- Transport at room temperature.

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Skin

- Cleanse the skin with alcohol.
- Collect epidermal scales with a scalpel at the active border of the lesion.
- Place the specimens in a dry sterile container.
- Transport at room temperature.

Mouth, Throat or Vaginal

- Collect specimen on swab
- Place in non-gel Aimes transport media
- Transport at room temperature or refrigerated.

Fungus Culture

Scalp and Hair

- Scrape the scalp with a blunt scalpel.
- Collect the basal portion of the infected hair.
Note: Infected hairs may be selected by placing the patient under a UV light (Wood's lamp). Hairs infected with some dermatophytes will fluoresce under UV light. Hairs that are fluorescent, distorted or fractured should be cultured.
- Place hair in a dry sterile container.
- Transport at room temperature.
- The following specimens are also acceptable:
 1. Hair stubs
 2. Contents of plugged follicles
 3. Skin scales
 4. Hair plucked from the scalp with forceps.
- The following specimens are **not** acceptable:
 1. Cut hair

Nails

- Cleanse the nail with alcohol.
- Remove the outermost layer by scraping with a scalpel deep enough to obtain recently invaded nail tissue. (discard initial scrapings as they are usually contaminated)
- The following specimens are also acceptable:
 1. Clippings from any discolored or brittle parts of nail
 2. Deeper scrapings and debris under the edges of the nail
- Place specimen in a dry sterile container.
- Transport at room temperature.

Skin

- Cleanse the skin with alcohol
- With a scalpel or the edge of a glass slide, collect the active, peripheral edge of the lesion.
- Place the scales in a dry sterile container. Collect enough scraping to cover the head of a thumbtack.
- Transport at room temperature.

Other Sources (Submit fluid or culture swab)

1. Bone Marrow – collect 0.5 ml aspirated bone marrow
2. Blood – collect 2 sets of blood cultures (40 ml of blood). Transport at room temperature.
3. Corneal scraping, intraocular aspiration, or biopsy obtained by an ophthalmologist.

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4. Sputum – collect first early morning deep cough sample after patient's teeth are brushed and the mouth is well rinsed (24 hr. specimens are not satisfactory). Transport refrigerated.
5. Subcutaneous Specimens (wound, abscess, lesion, pus, drainage) - For suppurative lesions of the deep skin and subcutaneous tissue, where pus may be loculated within abscess or is exuding from deep sinus tracts, aspiration with a sterile needle and syringe should be attempted. The material should be placed in an anaerobic transport at room temperature. In addition to a fungus culture, both anaerobic and aerobic cultures should be performed, submitted in anaerobic transport media. The former being necessary to recover the anaerobic branching filamentous bacteria belonging to the genus Actinomyces.
6. Tissue – obtained in surgery. Place on sterile 4x4 gauze or in sterile container and transport immediately at room temperature.
7. Body Fluids and Exudates – samples are usually obtained by aspiration with a sterile needle and syringe. Place in sterile screw tap container and transport at room temperature.
8. Urine – collect first morning “mid-stream” specimen. Do not obtain urine samples from a collection bag or bedpan. Transport refrigerated.
9. Stool – rarely worth culturing – growth of a large amount of yeast has possible significance, but only in indicating a lack of normal flora.

WOUND CULTURE COLLECTION

Abscess (Wounds)

Tissue or fluid is always superior to a swab specimen.

- Remove surface exudate by wiping with sterile saline or 70% alcohol.
For open abscess:
 1. Aspirate, if possible, or pass the swab deep into the lesion and firmly sample lesion's advancing edge.
 2. When a swab is used, place swab into anaerobic transport media. Leave aspirate in securely capped syringe if delivered within 30 minutes.For closed abscess:
 1. Aspirate abscess wall material with needle and syringe.
 2. Aseptically transfer all material into an anaerobic transport media or leave in the securely capped syringe if delivered within 30 minutes.
- Transport immediately at room temperature.

Note: A sample from the base of the lesion and a sample from the abscess wall are most productive. Sampling of the surface area can introduce colonizing bacteria not involved in the infectious process.

Burn

- Clean and debride the wound prior to specimen collection. A 3-4 mm punch biopsy is optimum.
- Process for aerobic culture only. Send in a sterile container or Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Note: Surface cultures of burns may be misleading.

Cellulitis

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- Cleanse site by wiping with sterile saline or 70% alcohol.
- Aspirate the area of maximum inflammation (commonly the center rather than the leading edge) with a fine needle and syringe.
- Draw small amount of sterile saline into syringe and aspirate into sterile screw-cap tube or place in Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Decubitis Ulcer

Note: A decubitis swab provides little clinical information; culturing is discouraged. A tissue biopsy sample or needle aspirate is the specimen of choice.

- Cleanse the surface with sterile saline.
- If sample biopsy is not available, vigorously swab the face of the lesion. A swab specimen is not the specimen of choice.
- Place the swab in Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Catheter Tip Cultures

Short catheters (2-3 inches)

- Decontaminate the skin at the catheter site.
- Aseptically remove the catheter. Cut the catheter at the skin interface point using sterile technique.
- If the specimen will arrive at NPL within 30 minutes: place the catheter segment in a sterile wide-mouth container. If transport is delayed, place catheter tip in Aimes or Stuarts transport media, but preferably not in the gel transport. Remove swab from cap and discard.
- Transport immediately at room temperature.

Long catheters (8-24 inches)

- Decontaminate the skin at the catheter site.
- Aseptically remove the catheter. Submit two segments for analysis. Cut a 2 inch segment of the catheter that was within the blood vessel, using sterile technique.
- If the specimen will arrive at NPL within 30 minutes: place the segment in a sterile wide-mouth container. Cut a second 2 inch segment of the catheter from the skin interface. Place the segment in a sterile wide-mouth container. If transport is delayed, place catheter tip in Aimes or Stuarts transport media, but preferably not in the gel transport. Remove swab from cap and discard.
- Label the containers appropriately.
- Transport immediately at room temperature.

NASOPHARYNGEAL SWAB

Nylon flocked swabs in conjunction with Universal Transport medium (UTM) is the specimen of choice for collection of respiratory virus specimens. A nasal wash or aspirate is also acceptable.

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Nylon Flocked Nasopharyngeal Swabs absorb and release more sample volume

Collection Procedure:

1. Gently pass the flocked swab through the nose and into the posterior nasopharynx.
2. Rotate the swab on the nasopharyngeal membrane 5-6 times and allow it to remain in place for 10-15 seconds.
3. Remove the swab and repeat the procedure in the other nare using the same swab.
4. Remove cover from the UTM, place the swab in the liquid and snap the swab shaft off at the score and leave the swab inside the vial. Replace the cap; be sure to tighten securely to prevent leakage.
5. Transport immediately at room temperature. If a delay in transporting, send refrigerated.

Note: Specimens received on large (culture type) swabs are assumed to be from the nose and not the nasopharynx regardless of the source given on the specimen label.

NASOPHARYNGEAL ASPIRATE

- Attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter. Turn on suction and adjust to suggested pressure.
- Without applying suction, inset catheter into the nose, directed posteriorly and toward the opening of the external ear. **NOTE:** Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior nares and external opening of the ear.
- Apply suction. Using a rotating movement, slowly withdraw the catheter.
- Place specimen in sterile container.
- Transport immediately at room temperature. If delay in transporting, send refrigerated.

NASOPHARYNGEAL WASH

- Suction 3.0-5.0 mL of sterile saline into a new sterile bulb.
- With the patient's head tipped back slightly, insert bulb into one nostril until nostril is occluded.
- Instill saline into one nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
- Empty bulb into dry, sterile specimen container.

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- Transport immediately at room temperature. If delay in transporting, send at refrigerator temperature.

OCCULT BLOOD COLLECTION

A plastic collection “hat” is placed over the rim of the toilet seat to collect the specimen.

- Open the front side of the occult blood collection card (the side that contains the patient information).
- Using a wooden application stick, collect a small stool sample on one end of an applicator stick.
- Apply a thin sample of feces inside Box A.
- Reuse the applicator stick to obtain a second sample from a different part of the stool.
- Apply a thin sample inside Box B.
- Discard applicator stick in a waste container.
- Close and secure the front flap by securing under tab.

PINWORM COLLECTION

Because of the migratory habits of the female pinworm, specimens are best obtained a few hours after the person has retired, or in the morning immediately upon rising before bathing or bowel movement. The room should remain darkened while the collection is being done.

- Hold the paddle by the cap and remove it from the tube. Separate the buttocks and press the sticky surface against several areas of the perianal region.
- Replace the paddle in the tube. (If collecting more than one specimen, they should be collected on consecutive days.)
- If paddle is not available, use clear tape. Press the sticky side of the clear tape to the perianal region. After collection, press sticky side on a glass slide. Do not use frosted tape for specimen collection.
- Transport at room temperature.

RESPIRATORY CULTURE COLLECTION

Nose

- Seat the patient comfortably. Patient’s head should be inclined from vertical for proper specimen recovery.
- Insert a sterile culture swab into one nostril, up and over the floor of the nostril. (Approximately 2 cm into the nostril).
- Rotate the culture swab gently and allow the swab to remain for at least 10 seconds and up to 30 seconds. Remove the culture swab gently.
- Using the same culture swab, repeat the procedure in the other nostril.
- Place the swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

Sputum

Assure patient cooperation to get an adequate specimen. The laboratory will determine numbers of squamous epithelial cells present, to determine specimen adequacy.

- Ask patient to rinse mouth with water before attempting collection.

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- Instruct the patient to cough deeply to produce lower respiratory tract specimen (not post nasal fluid). Collect in a sterile container.
If the patient is unable to produce sputum, induce sputum by postural drainage, saline nebulization, or chest percussion.
- Transport the specimen in the sterile container promptly.
- If a delay in transport, swab the specimen and place in Aimes or Stuart's transport media. Transport refrigerated.

STOOL COLLECTION

Pathogens routinely cultured for are: Salmonella, Shigella, Ecoli 0157, Aeromonas, and Campylobacter. If Vibrio, Yersinia or Shiga-Toxin are suspected, please request appropriately.

- Collect specimen in a clean container. Do not submit feces contaminated with urine or toilet water.
- Transfer specimen into a clean, dry container.
- Transport fresh feces at room temperature within 1 hour of collection. If transport delay is unavoidable, place the specimen in an appropriate preservative immediately after collection.
 - Liquid Cary-Blair for bacterial culture or O&P by immunoassay (OVP), transport refrigerated.
 - Apacor Parasep SF (green topped transport) for OPFA (patient with foreign travel history). Follow package insert instruction for proper sample quantity. Transport at room temperature.
 - Liquid Cary-Blair for Giardia Antigen (GAT) or Cryptosporidium EIA (CRYPT) test. Transport refrigerated.

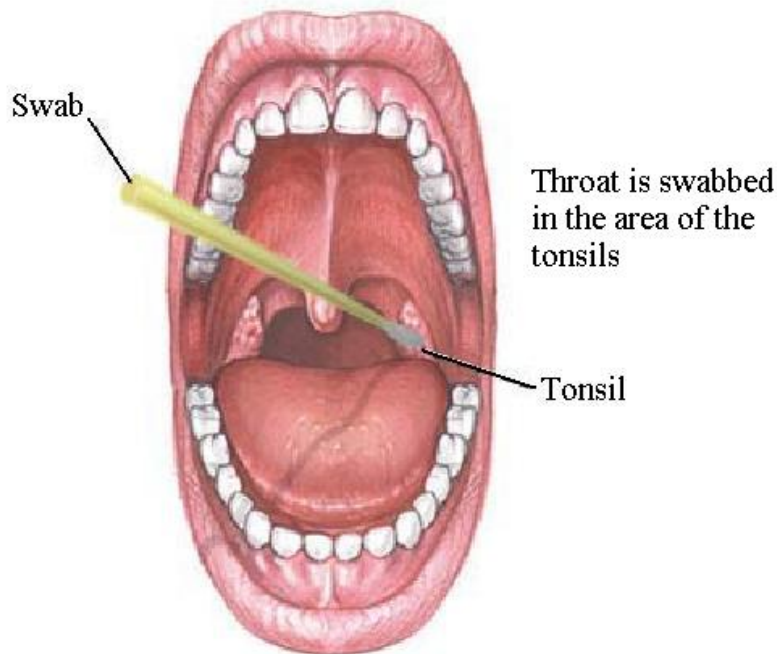
Note: For ova and parasite testing, radiologic examination using bismuth or barium should be avoided 7-10 days prior to collection. If three specimens are being provided, wait 48 hours between collection. Do not routinely perform stool cultures for patients whose length of stay was > 3 days.

THROAT CULTURE COLLECTION

- Use a dacron or rayon culture swab.
- Use a tongue blade and a good light source to ensure good visualization.
- Reach behind the uvula and swab:
 1. Both tonsillar fauces, and
 2. the posterior pharynx, and
 3. any ulceration, exudate, lesion, or area of inflammation.
- Place the swab into Aimes or Stuarts transport media.
- Transport at room temperature or refrigerated.

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URINE COLLECTION

Routine Urine – Male and Female

For instructions on patient collection, see clean catch urine directions.

- Refrigerate within 10 minutes of collection.
- Transport the sterile specimen cup or if shipping, transfer specimen into the BD boric acid urine transport tube.

Note: Do not submit the following:

- a. A sample taken from a larger container, such as a urinal or bedpan.
- b. A specimen brought from home.

Indwelling Catheter Urine

Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.

- Clean the catheter with an alcohol pad.
- Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
- Transfer the urine to a sterile specimen cup. If shipping is delayed, transfer specimen into the BD boric acid transport tube.
- Transport refrigerated.

Note: Do not culture a catheter tip because it may be contaminated as it is removed from the urethra.